



172

PROGRESS IN
BRAIN RESEARCH

Serotonin-Dopamine Interaction Experimental, Clinical and Therapeutic Evidence

EDITED BY
GIUSEPPE DI GIOVANNI
VINCENZO DI MATTEO
ENNIO ESPOSITO

PROGRESS IN BRAIN RESEARCH

VOLUME 172

SEROTONIN–DOPAMINE INTERACTION: EXPERIMENTAL EVIDENCE AND THERAPEUTIC RELEVANCE

EDITED BY

GIUSEPPE DI GIOVANNI

Dipartimento di Medicina Sperimentale,

Sezione di Fisiologia Umana “G. Pagano”, Università degli Studi di Palermo, Palermo, Italy

VINCENZO DI MATTEO and ENNIO ESPOSITO

Istituto di Ricerche Farmacologiche “Mario Negri”,

Consorzio Mario Negri Sud, Santa Maria Imbaro, Chieti, Italy



ELSEVIER

AMSTERDAM – BOSTON – HEIDELBERG – LONDON – NEW YORK – OXFORD
PARIS – SAN DIEGO – SAN FRANCISCO – SINGAPORE – SYDNEY – TOKYO

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
Linacre House, Jordan Hill, Oxford OX2 8DP, UK

First edition 2008

Copyright © 2008 Elsevier B.V. All rights reserved

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at <http://www.elsevier.com/locate/permissions>, and selecting *Obtaining permission to use Elsevier material*

Notice

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

ISBN: 978-0-444-53235-0 (this volume)

ISSN: 0079-6123 (Series)

For information on all Elsevier publications
visit our website at books.elsevier.com

Printed and bound in Hungary

08 09 10 11 12 10 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries

www.elsevier.com | www.bookaid.org | www.sabre.org

ELSEVIER BOOK AID International Sabre Foundation

List of contributors

- J.M.C. Alfaro, Laboratorio de Neurociencias, División de Estudios de Posgrado, Facultad de Ciencias Médicas y Biológicas “Dr. Ignacio Chávez”, Universidad Michoacana de San Nicolás de Hidalgo, Morelai, Mich., Mexico
- P. Anguiano-Rodríguez, Laboratorio de Neurofisiología Experimental, Centro de Investigación Biomédica de Michoacán, Instituto Mexicano del Seguro Social, Morelai, Mich., Mexico
- M.H. Baumann, Clinical Psychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, DHHS, Baltimore, MD, USA
- A. Benigno, Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, Università di Palermo, Corso Tuköry, Palermo, Italy
- K.A. Berg, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX, USA
- A. Björklund, Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden
- B.E. Blough, Chemistry and Life Sciences Group, Research Triangle Institute International, Research Triangle Park, NC, USA
- V. Boulougouris, Department of Experimental Psychology and the Behavioural and Clinical Neuroscience Institute (BCNI), University of Cambridge, Cambridge, UK
- J.M. Brotchie, Toronto Western Research Institute, Toronto, Ont., Canada
- M.J. Bubar, Center for Addiction Research, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA
- R.J. Carey, Research and Development (151), VA Medical Center and SUNY Upstate Medical University, Syracuse, NY, USA
- T. Carlsson, Neurobiology Unit, Wallenberg Neuroscience Center; Brain Repair and Imaging in Neural Systems Unit, Section for Neuroscience, Department of Experimental Medical Science, Lund University, Lund, Sweden
- M. Carta, Neurobiology Unit, Wallenberg Neuroscience Center; Brain Repair and Imaging in Neural Systems Unit, Section for Neuroscience, Department of Experimental Medical Science, Lund University, Lund, Sweden
- R. Chuang, Movement Disorders Clinic, University of Toronto, Toronto Western Hospital, Toronto, Ont., Canada
- W.P. Clarke, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX, USA
- I.W. Craig, MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre, Institute of Psychiatry, King's College London, Denmark Hill, London, UK
- G. Crescimanno, Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, Università di Palermo, Corso Tuköry, Palermo, Italy
- K.A. Cunningham, Center for Addiction Research, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA
- J. de Almeida, Departament de Neuroquímica, Institut d'Investigacions Biomediques de Barcelona, Consejo Superior de Investigaciones Científicas (CSIC), IDIBAPS Rossello, Barcelona, Spain

- G. Di Giovanni, Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, Università di Palermo, Corso Tuköry, Palermo, Italy
- V. Di Matteo, Istituto di Ricerche Farmacologiche “Mario Negri”, Consorzio “Mario Negri” Sud, Santa Maria Imbaro, Chieti, Italy
- U.M. D’Souza, MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre, Institute of Psychiatry, King’s College London, Denmark Hill, London, UK
- E. Esposito, Istituto di Ricerche Farmacologiche “Mario Negri”, Consorzio “Mario Negri” Sud, Santa Maria Imbaro, Chieti, Italy
- A. Feria-Velasco, Laboratorio de Neurobiología Celular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Jal., México
- P.J. Fletcher, Centre for Addiction and Mental Health; Department of Psychology; Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada
- S.H. Fox, Movement Disorders Clinic, University of Toronto, Toronto Western Hospital, Toronto, Ontario, Canada
- A. Friedman, Neuropharmacology Laboratory, The Mina and Everard Goodman Faculty of Life Sciences and the Leslie and Susan Gonda (Goldshmiel) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, Israel
- I. González-Burgos, Laboratorio de Psicobiología, División de Neurociencias, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social; Laboratorio de Neurobiología Celular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Jal., México
- J.A. Harvey, Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, USA
- G.A. Higgins, Bioquest Innovations Inc., Toronto, Ontario, Canada
- M. Huang, Department of Psychiatry, Vanderbilt University School of Medicine, The Psychiatric Hospital at Vanderbilt, Nashville, TN, USA
- J.P. Huston, Institute of Physiological Psychology, University of Düsseldorf, Düsseldorf, Germany
- H. Jantos, Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Montevideo, Uruguay
- P.E. Keck Jr., Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- D. Kirik, Brain Repair and Imaging in Neural Systems Unit, Section for Neuroscience, Department of Experimental Medical Science, Lund University, Lund, Sweden
- T. Kuroki, Clinical Research Division, National Hospital Organization, Hizen Psychiatric Center, Kanzaki, Saga, Japan
- A.D. Lê, Centre for Addiction and Mental Health; Department of Pharmacology; Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada
- X. Li, University of Ottawa Institute of Mental Health Research, Ottawa, Ont., Canada
- M. Lindström, Section of Psychiatry, Department of Clinical Neuroscience, University Hospital of Lund, Lund, Sweden
- A.D. Logue, Cincinnati Veterans Affairs Medical Center; Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- M.Á. Loópez-Vázquez, Laboratorio de Neurofisiología Experimental, Centro de Investigación Biomédica de Michoacán, Instituto Mexicano del Seguro Social, Morelai, Mich., Mexico
- J.-C. Maillet, University of Ottawa Institute of Mental Health Research, Ottawa, Ont., Canada
- R.K. McNamara, Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- H.Y. Meltzer, Department of Psychiatry, Vanderbilt University School of Medicine, The Psychiatric Hospital at Vanderbilt, Nashville, TN, USA

- G. Mengod, Departament de Neuroquímica, Institut d'Investigacions Biomediques de Barcelona, Consejo Superior de Investigaciones Científicas (CSIC), IDIBAPS Rosello; Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain
- K.A. Michelsen, Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University; European Graduate School of Neuroscience (EURON), 6200 MD Maastricht, The Netherlands
- J.M. Monti, Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Montevideo, Uruguay
- C.P. Müller, MRC-SGDP Center, Institute of Psychiatry, King's College London, De Crespigny Park, London, UK
- A. Munoz, Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden
- N. Nagao, Clinical Research Division, National Hospital Organization, Hizen Psychiatric Center, Kanzaki, Saga, Japan
- T. Nakahara, Clinical Research Division, National Hospital Organization, Hizen Psychiatric Center, Kanzaki, Saga, Japan
- R.D. Oades, Biopsychology Group, Clinic for Child and Adolescent Psychiatry and Psychotherapy, University of Duisburg-Essen, Essen, Germany
- M.E. Olvera-Cortés, Laboratorio de Neurofisiología Experimental, Centro de Investigación Biomédica de Michoacán, Instituto Mexicano del Seguro Social; Laboratorio de Neurociencias, División de Estudios de Posgrado, Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mich., Mexico
- J.M. Palacios, Universitat de Barcelona, Barcelona, Spain
- M. Pierucci, Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, Santa Maria Imbaro, Chieti, Italy
- J. Prickaerts, Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, European Graduate School of Neuroscience (EURON), 6200 MD Maastricht, The Netherlands
- G. Remington, Department of Psychiatry, Faculty of Medicine, University of Toronto; Medication Assessment Program for Schizophrenia (MAPS) Clinic, Schizophrenia Program, Centre for Addiction and Mental Health (CAMH), Toronto, Ont., Canada
- N.M. Richtand, Cincinnati Veterans Affairs Medical Center, Psychiatry Service (V116A); Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- R.B. Rothman, Clinical Psychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, DHHS, Baltimore, MD, USA
- E. Ryding, Department of Clinical Neurophysiology, Karolinska Hospital, Huddinge, Stockholm; Section of Clinical Neurophysiology, Department of Neuroscience, University Hospital of Lund, Lund, Sweden
- U. Spampinato, Université Victor Segalen Bordeaux 2, Centre de Recherche Inserm U862, Institut Francois Magendie, Bordeaux Cedex, France
- T.D.L. Steeves, Division of Neurology, University of Toronto and the Morton and Gloria Shulman Movement Disorders Centre, Toronto Western Hospital, Ontario, Canada
- H.W.M. Steinbusch, Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University, European Graduate School of Neuroscience (EURON), 6200 MD Maastricht, The Netherlands
- S.M. Strakowski, Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- L. Träskman-Bendz, Section of Psychiatry, Department of Clinical Neuroscience, University Hospital of Lund, Lund, Sweden

- E. Tsaltas, Experimental Psychology Laboratory, Department of Psychiatry, Athens University Medical School, Eginition Hospital, Athens, Greece
- J.A. Welge, Department of Psychiatry; Center for Biostatistical Services, University of Cincinnati College of Medicine, Cincinnati, OH, USA
- M.D. Wood, Psychiatry Centre of Excellence for Drug Discovery, Department of Biology, GlaxoSmithKline, New Frontiers Science Park, Harlow, Essex, UK
- P.B. Wren, Psychiatry Centre of Excellence for Drug Discovery, Medicines Research Centre, Verona, Italy
- G. Yadid, Neuropharmacology Laboratory, The Mina and Everard Goodman Faculty of Life Sciences and the Leslie and Susan Gonda (Goldshmid) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan, Israel
- X. Zhang, University of Ottawa Institute of Mental Health Research, University of Ottawa, Ottawa, Ont., Canada
- Y. Zhang, University of Ottawa Institute of Mental Health Research, Ottawa, Ont., Canada

Preface

First and foremost, we give our heartfelt thanks to Giuseppe, who conceived the original idea for this book and whose passion and tireless hard work lead to its realization. Moreover, he has always been supportive throughout the highs and lows of editing. The choice of text has been made assuming that the reader has maintained an interest in serotonin and dopamine, a field complicated, vast, and at present, probably one of the richest in scientific literature, and is now keen to embark on a more in-depth study of their interaction. With this in mind, we have sought to offer as varied a picture as possible, by including different points of view, together with different aspects of the serotonin–dopamine interactions in both physiological and pathological phenomena. New evidence has recently emerged thanks to the development of new techniques in molecular biology, genetics, single cell and membrane physiology, clinical neurology, neuropsychiatry and brain imaging *in vivo*. In this volume, we seek to provide a systematic overview of these recent developments in our understanding of the chemical neuroanatomy of the interaction of serotonin and dopamine from a functional perspective.

The selection starts, after an overview of the topic, by reviewing the interactions between these two monoamines from an electrophysiological and neurochemical point of view, in different brain areas. Thereafter, the polymorphisms in monoamine neurotransmitter pathway genes and the involvement in the physiopathology of schizophrenia, depression, drug abuse, attention-deficit hyperactivity disorder, Parkinson's disease, Tourette's syndrome and in cognition processes are examined from different perspectives. We will feel that our labour has been worthwhile if any reader is stimulated to further study of this rich literature or is inspired to research this important and fascinating scientific field. Moreover, both pharmacologists looking for new systems for developing drugs, and neuroscientists, whether researchers or advanced students, will find this important work useful.

While covering the latest research, for obvious reasons this book cannot be exhaustive and we are sorry indeed that it has been impossible to include a number of authors of obvious merit. The selection is intended, indeed, to be merely a very varied foretaste of contemporary research on the subject. Therefore, we would like to see this book as an ongoing project, in which future editions will follow the developments in the field. We consider however, that the varied selection has a certain unity as physiologic, pathologic and psychological themes run through the book and supply the logical connections between the various authors.

The editors wish to thank all the authors who have responded so willingly to contribute their time and expertise in preparing their individual chapters to a consistently high standard. Our warmest thanks go to Hilary Rowe, Elsevier publishing editor, who believed in the potential of this book and the importance of the messages it conveys and Maureen Twaig who has helped to drive the book's development and eventual publication. Our thanks go to everyone who has, in same way, contributed to the realization of this book, notably Samantha, Caterina, Christopher, Barbara, colleagues at the '*Cosorzio Mario Negri Sud*' and the *University of Palermo*. And finally, we are deeply indebted to Dr. Clare Austen for a very insightful and helpful reading and reviewing of the English style of these manuscripts. She worked hard, sometimes at very short notice and put up with the editors' minor inefficiencies.

Giuseppe Di Giovanni
Vincenzo Di Matteo
Ennio Esposito
Italy, April 2008

Dedication

In loving memory of Giulio Di Giovanni, a wonderful father

CHAPTER 1

Serotonin–dopamine interaction: an overview

Ennio Esposito^{1,*}, Vincenzo Di Matteo¹ and Giuseppe Di Giovanni²

¹*Istituto di Ricerche Farmacologiche “Mario Negri”, Consorzio “Mario Negri”, Via Nazionale,
66030 Santa Maria Imbaro, Chieti, Italy*

²*Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, “G. Pagano”, Università di Palermo,
Corso Tuköry 129, 90134 Palermo, Italy*

Abstract: Central serotonergic and dopaminergic systems play a critical role in the regulation of normal and abnormal behaviours. Moreover, recent evidence suggests that the dysfunction of dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) neurotransmission might underlie the pathophysiology of neuropsychiatric disorders, including depression, schizophrenia, attention deficit hyperactivity disorders, drug abuse, Gilles de la Tourette’s syndrome and Parkinson’s disease.

Keywords: serotonin; dopamine; serotonin receptors; substantia nigra; ventral tegmental area; neuropsychiatric disorders

The interaction between the two amines in the brain, in normal and pathological conditions, is an intriguing research field and a better understanding will provide new pharmacological approaches for the treatment of several neuropsychiatric disorders. An extensive scientific literature has investigated the role of serotonin (5-hydroxytryptamine, 5-HT) in the control of central dopamine (DA) systems, and their dysfunction in pathological conditions. In neuropsychiatric disorders, the involvement of 5-HT and DA has been indicated and singled out and new therapeutic approaches suggested. Nevertheless, the interaction between 5-HT and DA systems is far from being completely revealed.

The interaction between 5-HT and DA in the brain is a research topic that has raised the interest of many scientists working in the field of

neuroscience since the first demonstration of the presence of monoamine-containing neurons in the mid-1960s. Thus, a seminal work by Fuxe (1965) reported the presence of 5-HT-containing terminals in both the substantia nigra (SN) and the ventral tegmental area (VTA). It was subsequently found that serotonergic nerve terminals originating from the dorsal raphe nucleus (DRN) innervated both the SN and the VTA (Steinbusch, 1981), whereas 5-HT fibres arising from median raphe nucleus (MNR) innervated the VTA but not the SN (Fibiger and Miller, 1977; Azmitia and Segal, 1978). Based on those anatomical studies, several investigators began to study the behavioural, biochemical and electrophysiological relevance of the neuroanatomical findings. Further impetus was given to this research by the increasing evidence that most psychotropic drugs, including antidepressants, antipsychotics, opioids, anxiolytics and psychostimulants, exerted their pharmacological actions by interfering with serotonergic and dopaminergic transmission. An

*Corresponding author. Tel.: +39 0872 570274;
Fax: +39 0872 570416; E-mail: esposito@negrissud.it

almost parallel and concomitant progress was made in the identification, characterization and cloning of 5-HT receptor subtypes (Boess and Martin, 1994).

Early studies showed that experimental manipulations aimed at decreasing central 5-HT function, such as selective nerve lesions by neurotoxin, inhibition of 5-HT synthesis or 5-HT receptor blockade, tended to potentiate the behavioural and neurochemical effects of drugs such as amphetamine and related compounds, enhancing the dopaminergic transmission, thus leading to the conclusion that central serotonergic systems inhibit DA functions (Samanin and Garattini, 1975). The interest of our laboratory in investigating 5-HT/DA interaction sprang from these early studies. Our approach was based on *in vivo* electrophysiological and neurochemical techniques. The experimental data gathered over a period of almost 20 years lead to the conclusion that several 5-HT receptor subtypes, including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT₃ and 5-HT₄ receptors, act to facilitate neuronal DA function and release, while the 5-HT_{2C} receptor mediates an inhibitory effect of 5-HT on the basal electrical activity of dopaminergic neurons and on DA release.

A detailed analysis and discussion of the above-mentioned data are reported in this book. Of particular interest is the finding that 5-HT_{2A} and 5-HT_{2C} receptors exert opposite actions on dopaminergic activity, although they belong to the same receptor family, and share almost identical cellular transduction mechanisms. One possible mechanism of this opposite action is the intermediation of a γ -aminobutyric acid (GABA)-ergic neuron in the inhibitory effect of 5-HT_{2C} receptors on DA function. Based on this hypothesis, 5-HT_{2A} receptor stimulation would cause a direct excitation of DA-containing neurons, whereas activation of 5-HT_{2C} receptors would indirectly inhibit these neurons by stimulating a GABA-containing interneuron (Di Giovanni et al., 2001; Di Matteo et al., 2001). However, although early neuroanatomical data showing that 5-HT_{2C} receptors are restricted to GABA-containing neurons in the SN (Eberle-Wang et al., 1997) strengthened this hypothesis, it was recently found that in the VTA, 5-HT_{2C} receptor protein is expressed on both DA

and GABA-containing neurons (Bubar and Cunningham, 2007). Thus, there are several possible anatomical sites of 5-HT/DA interaction: it may be at the level of brainstem where SN and VTA are located, and/or at the level of projection areas of nigrostriatal and mesocortico-limbic dopaminergic systems, including the striatum, the nucleus accumbens and the prefrontal cortex. The interaction might be either direct (i.e. mediated by 5-HT receptors located on the cell body and/or on nerve terminals of DA neurons) or indirect (i.e. by 5-HT receptors located on interneurons impinging on DA neurons and/or by short or long feed-back loops originating from innervated brain areas).

It is interesting to note that a number of behavioural studies are consistent with electrophysiological and neurochemical findings, in that 5-HT_{2C} receptor antagonists were found to potentiate the effects of several psychostimulant drugs including cocaine, nicotine and amphetamine-like compounds, whereas 5-HT_{2A} receptor antagonists had the opposite effect (Bubar and Cunningham, 2008). Moreover, 5-HT_{2C} receptor agonists reduce the locomotor and rewarding effects of several drugs of abuse, including nicotine, ethanol, cocaine and amphetamine-like drugs. It is noteworthy that the locomotor and rewarding effects of cocaine were clearly potentiated in 5-HT_{2C} receptor null mutant mice (Rocha et al., 2002). Therefore, an important aspect of 5-HT/DA interaction relates to the possibility of using 5-HT_{2C} receptor agonists in the therapy of addictive disorders. In this respect, it is important to note that several new selective and potent 5-HT_{2C} receptor agonists have been developed, which might be useful to treat disturbances of consummatory behaviours such as overeating and drug addiction, which are now considered related disorders. An intriguing aspect of this problem is the recent finding that the tumour suppressor enzyme PTEN (phosphatase and tensin homologue deleted on chromosome 10) directly interacts with a region in the third intracellular loop (3L4F) of 5-HT_{2C} receptors in the rat VTA. PTEN limits agonist-induced 5-HT_{2C} receptor phosphorylation via its protein phosphatase activity. Systemic or intra-VTA application of the

interfering peptide Tat-3L4F is capable of disrupting PTEN coupling with 5-HT_{2C} in the rat VTA, resulting both in a suppression of the increased firing rate of VTA dopaminergic neurons induced by Δ^9 -tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, and in a blockade of the conditioned place preference induced by THC and nicotine (Ji et al., 2006). This represents a very good example of integration of classical pharmacology with up-to-date molecular biology techniques.

Another important characteristic of the 5-HT_{2C} receptor subtype is that it undergoes post-transcriptional editing. Thus, the 5-HT_{2C} receptor is the only seven transmembrane domain receptor to date found to undergo the post-transcriptional process of mRNA editing, which generates unique isoforms of proteins in a cell- and/or tissue-specific manner. mRNA transcripts of the rat and human 5-HT_{2C} receptor undergo adenosine-to-inosine editing events at five sites which encompass amino acids 156–160 within the putative second intracellular domain of the encoded human receptor, resulting in the production of fourteen 5-HT_{2C} receptor isoforms (Berg et al., 2005). There is increasing evidence that some of these isoforms might be linked to a higher probability of developing depression and psychosis.

The chapters of this book are devoted to the analysis of the relevance of 5-HT/DA interaction in learning and memory, the sleep/wake cycle, attention and impulsivity, and the pathophysiology of several neuropsychiatric disorders, including depression, schizophrenia and other psychotic disorders, drug abuse, Gilles de la Tourette's syndrome, attention deficit hyperactivity disorders (ADHD), Parkinson's disease, Alzheimer's disease, dyskinesias and motor tics. The detailed knowledge of 5-HT/DA interaction would also help in understanding the mechanism of action of most psychotropic drugs, including antidepressant, antipsychotic and anti-addictive drugs that is thought to be mediated, at least in part, via the 5-HT system. 5-HT research is now more than 50 years old, and it has generated a wealth of therapeutic agents, some of which have had a major impact on disease management. Not surprisingly, 5-HT receptors and transporters

continue to be a major focus of CNS drug discovery. In fact, of the top CNS drugs by total sales, most modulate 5-HT neurotransmission (Segest, 2007). Furthermore, the selective 5-HT reuptake inhibitors (SSRIs) are among the most widely prescribed drugs for treating depression and a variety of other disorders including anxiety and social phobia. But we are a long way from a serotonergic therapeutic intervention for other neuropsychiatric diseases.

5-HT receptor research has generated detailed information on the molecular biology and regional and cellular localization of these receptors. A major challenge now is to utilize this knowledge to develop receptor-specific drugs and use the information gained to better treat central nervous system disorders. In addition, further clarification of the role of 5-HT transmission in the pathophysiology of neuropsychiatric disorders is required, since the overall picture is still confusing. It is likely that 5-HT_{2C} ligands are promising candidates (Di Giovanni et al., 2006; Rosenzweig-Lipson et al., 2007). Moreover, there are also many avenues that remain unexplored, so there are undoubtedly further advances to be made. The intensive research in medicinal chemistry will help this field of investigation. In fact, more selective ligands for 5-HT receptors are currently produced. In the future, the use of such selective ligands, especially agonists of 5-HT receptors, would certainly be helpful in determining their functional importance and their involvement in the pathogenesis of diseases, not exclusively of the CNS. This has a particular relevance for those disorders such as Parkinson's disease that are still fatal and for which at present there is no cure (Esposito et al., 2007; Di Giovanni, 2008). However, it should be kept in mind that although selective receptor ligands are an important and indispensable research tool, they rarely happen, in practice, to be drugs. Many questions need to be answered before we can truly understand how these 5-HT receptors regulate DA neuronal activity in the brain. The challenge ahead is to build on this foundation and keep up this engaging adventure: the interaction between 5-HT and DA systems is far from being completely revealed.

References

- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179: 641–659.
- Berg, K.A., Harvey, J.A., Spampinato, U. and Clarke, W.P. (2005) Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. *Trends Pharmacol. Sci.*, 26: 625–630.
- Boess, F.G. and Martin, I.L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology*, 33(3/4): 275–317.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Bubar, M.J. and Cunningham, K.A. (2008) Prospects for serotonin 5-HT_{2R} pharmacotherapy in psychostimulant abuse (Chapter 16 of this book).
- Di Giovanni, G. (2008) Will it ever become possible to prevent dopaminergic neuronal degeneration? *CNS Neurol. Disord. Drug Targets*, 7(1): 28–44.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) *m*-Chlorophenylpiperazine excites non-DA-ergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience*, 103: 111–116.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006) Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT_{2C} receptor be a new target for therapeutic strategies? *Curr. Med. Chem.*, 13(25): 3069–3081.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22: 229–232.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.-F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384: 233–247.
- Esposito, E., di Matteo, V., Pierucci, M., Benigno, A. and Di Giovanni, G. (2007) Role of central 5-HT_{2C} receptor in the control of basal ganglia functions. In: Di Giovanni G. (Ed.), *The Basal Ganglia Pathophysiology: Recent Advances*. Transworld Research Network, Trivandrum, pp. 97–127.
- Fibiger, H.C. and Miller, J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience*, 2: 975–987.
- Fuxe, K. (1965) Evidence for the existence of moniamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta Physiol. Scand.*, 247(Suppl.): 39–85.
- Ji, S.P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R. and Zhang, X. (2006) Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat. Med.*, 12(3): 324–329.
- Rocha, B.A., Goulding, E.H., O'Dell, L.E., Mead, A.N., Coufal, N.G., Parsons, L.H. and Tecott, L.H. (2002) Enhanced locomotor, reinforcing, and neurochemical effects of cocaine in serotonin, 5-hydroxytryptamine 2C receptor mutant mice. *J. Neurosci.*, 22: 10039–10045.
- Rosenzweig-Lipson, S., Dunlop, J. and Marquis, K.L. (2007) Agonists as an innovative approach for psychiatric disorders. *Drug News Perspect.*, 20(9): 565–571.
- Samanin, R. and Garattini, S. (1975) The serotonergic system in the brain and its possible functional connections with other aminergic systems. *Life Sci.*, (8): 1201–1209.
- Segest, S. (2007) *CNS Market Trends, 2007 to 2010*. URCH Publishing, London.
- Steinbusch, H.W.M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4): 557–618.

CHAPTER 2

Serotonin control of central dopaminergic function: focus on in vivo microdialysis studies

Vincenzo Di Matteo^{1,*}, Giuseppe Di Giovanni², Massimo Pierucci¹ and Ennio Esposito¹

¹*Istituto di Ricerche Farmacologiche “Mario Negri”, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro, Chieti, Italy*

²*Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, “G. Pagano”, Università degli Studi di Palermo, 90134 Palermo, Italy*

Abstract: In this review, the functional interactions between serotonin (5-HT) and dopamine (DA) neuronal systems are discussed with the focus on microdialysis studies in the rodent brain (mainly rats). 5-HT by itself is involved both directly and indirectly via actions on complex neuronal circuitry, in the regulation of DA release through multiple 5-HT receptors, playing a critical role in the development of normal and abnormal behaviours. Recent evidence suggests that dysfunction of dopaminergic and serotonergic neurotransmitter systems contributes to various disorders including depression, schizophrenia, Parkinson’s disease and drug abuse. Here we summarize recent neurochemical works that have extensively explored the role of 5-HT receptors in the control of DA central systems in both basal and drug-induced conditions, using in vivo microdialytic techniques. Several 5-HT receptor subtypes, including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT₃ and 5-HT₄ receptors, act to facilitate DA release, while the 5-HT_{2C} receptor mediates an inhibitory effect of 5-HT on DA release. Taken together, neurochemical approaches using microdialysis can not only contribute to clarification of the physiological role of the serotonergic neuronal systems but may also be a powerful pharmacological approach for the development of therapeutic strategies to the treatment of depression, schizophrenia, Parkinson’s disease and drug abuse.

Keywords: 5-HT receptors; dopaminergic function; microdialysis; mesocorticolimbic system; nigrostriatal system; antidepressants; antipsychotics; drug addiction

Introduction

There is now an extensive scientific literature regarding the functional interaction between serotonin (5-HT) and dopamine (DA)-containing neurons in the brain. In some cases, the nature of these interactions depends on the baseline

activity of DA and/or 5-HT systems, and whether they are activated. In addition, most of the effects of 5-HT on DA neurons may be indirect, mediated via actions on complex neuronal circuitry, rather than direct effects on DA terminals. In recent years, research on this matter has been spurred by new acquisition of important insights on the molecular biology of 5-HT receptor subtypes and by the availability of 5-HT receptor knockout mice (Bonasera and Tecott, 2000; Hoyer et al., 2002).

*Corresponding author. Tel.: +39 0872 5701;
Fax: +39 0872 570416; E-mail: vdimatteo@negrisud.it

Central serotonergic and dopaminergic systems play an important role in regulating normal and abnormal behaviours (Koob, 1992; Roth et al., 1992; Fibiger, 1995). Moreover, dysfunctions of 5-HT and DA neurotransmission are involved in the pathophysiology of various neuropsychiatric disorders including schizophrenia, depression and drug abuse (Koob, 1992; Roth et al., 1992; Brown and Gershon, 1993; Fibiger, 1995). Thus, the development of a number of relatively selective pharmacological agents with agonist or antagonist activity at a specific 5-HT receptor subtype has allowed investigators to better understand the functional role of these receptors in the control of central DA-ergic function, as 5-HT widely contributes to the regulation of a number of behavioural and physiological processes involving both limbic, cortical and striatal DA pathways (Jenck et al., 1998; Di Matteo et al., 2001; Higgins and Fletcher, 2003; Giorgetti and Tecott, 2004; Alex and Pehek, 2007). Since microdialysis is an established technique for studying a wide range of low molecular weight substances in the brain extracellular fluid, we here summarize recent neurochemical works that have extensively explored the role of 5-HT receptors in the control of DA central systems in both basal and drug-induced conditions, using *in vivo* microdialytic techniques. Therefore, the physiology, pharmacology and anatomical distribution of the 5-HT receptors in the central nervous system (CNS), as well as experimental data regarding the effect of 5-HT selective agents on the neuronal chemistry of DA central pathways, will be reviewed in this chapter, which will be introduced by a brief description of the functional neuroanatomy of dopaminergic and serotonergic systems. Finally, the potential use of 5-HT agents in the treatment of depression, schizophrenia, Parkinson's disease (PD) and drug abuse will be also discussed.

Dopamine systems

DA-containing neurons of the ventral mesencephalon have been designated as A8, A9 and A10 cell groups: these neurons can be collectively designated as the mesotelencephalic DA system

(Dahlström and Fuxe, 1964). Historically, the mesolimbic DA system was defined as originating in the A10 cells of the ventral tegmental area (VTA) and projecting to structures closely associated with the limbic system. This system was considered to be separated from the nigrostriatal DA system, which originates from the more lateral substantia nigra (SN; A9 cell group) (Dahlström and Fuxe, 1964; Bannon and Roth, 1983; Roth et al., 1987; Kalivas, 1993; White, 1996).

The mesolimbic and mesocortical DA systems appear critically involved in modulation of the functions subserved by cortical and limbic regions such as motivation and emotional control as well as cognition (Le Moal and Simon, 1991). Substantial evidence indicates that the mesolimbic pathway, particularly in the DA cells innervating accumbal areas, is implicated in the reward value of both natural and drug reinforcers, such as sexual behaviour or psychostimulants, respectively (Di Chiara and Imperato, 1988; Koob, 1992). Furthermore, animal studies have shown that lesion of DA terminals in the nucleus accumbens induces hypo-exploration, enhanced latency in the initiation of motor responses, disturbances in organizing complex behaviours and inability to switch from one behavioural activity to another (Le Moal and Simon, 1991). Hence, the mesolimbic DA system seems important for acquisition and regulation of goal-directed behaviours, established and maintained by natural or drug reinforcers (Le Moal and Simon, 1991; Kiyatkin, 1995).

The medial prefrontal cortex (mPFC) is generally associated with cognitive functions including working memory, planning and execution of behaviour, inhibitory response control and maintenance of focused attention (Le Moal and Simon, 1991). In addition, the mesolimbic DA pathway is sensitive to a variety of physical and psychological stressors (Roth and Elsworth, 1995). Indeed, recent studies have indicated that stress-induced activation of the mesocortical DA neurons may be necessary for the behavioural expression of such stimuli (Morrow et al., 1999).

The nigrostriatal DA system, which originates from the SN (A9 cell group), is one of the best studied, because of its involvement in the pathogenesis of PD (Grace and Bunney, 1985).

In mammals, the SN is a heterogeneous structure that includes two distinct compartments: the substantia nigra pars compacta (SNc) and the substantia nigra pars reticulata (SNr). The SNc represents the major source of striatal DA and, as already mentioned, its degeneration causes PD. On the contrary, the SNr mainly contains γ -amino-*n*-butyric acid (GABA)-ergic neurons which constitute one of the major efferences of the basal ganglia (Grace and Bunney, 1985).

Serotonin systems

Virtually all parts of the CNS receive innervation from serotonergic fibres arising from cell bodies of the two main subdivisions of the midbrain serotonergic nuclei, the dorsal raphe (DR) and the median raphe (MR) (Azmitia and Segal, 1978; Van der Kooy and Attori, 1980; Steinbush, 1984; Hervé et al., 1987; Van Bockstaele et al., 1993, 1994; Moukhles et al., 1997). 5-HT-containing cell bodies of the raphe nuclei send projections to dopaminergic cells in both the VTA and the SN, and to their terminal fields in the nucleus accumbens, prefrontal cortex and striatum (Van der Kooy and Attori, 1980; Steinbush, 1984; Hervé et al., 1987; Van Bockstaele et al., 1993, 1994; Moukhles et al., 1997) (Fig. 1). Electron microscopy demonstrates the presence of synaptic contacts of [3 H]5-HT-labelled terminals with both dopaminergic and non-dopaminergic dendrites in all subnuclei of the VTA, and in the SNc and SNr (Hervé et al., 1987; Kalivas, 1993; Moukhles et al., 1997).

The precise nature of the interaction between 5-HT and DA has been difficult to elucidate, in that both inhibitory and excitatory roles for 5-HT have been suggested. However, these discrepancies may be attributable to the differential distribution and to the diverse functional roles of 5-HT receptor subtypes within the dopaminergic systems (Hoyer et al., 1994; Barnes and Sharp, 1999). Thus, much attention has been devoted to the role of 5-HT receptors in the control of central DA activity, because of their implication in the pathophysiology of the diseases that affect central DA systems, such as schizophrenia, depression, drug abuse, PD and others.

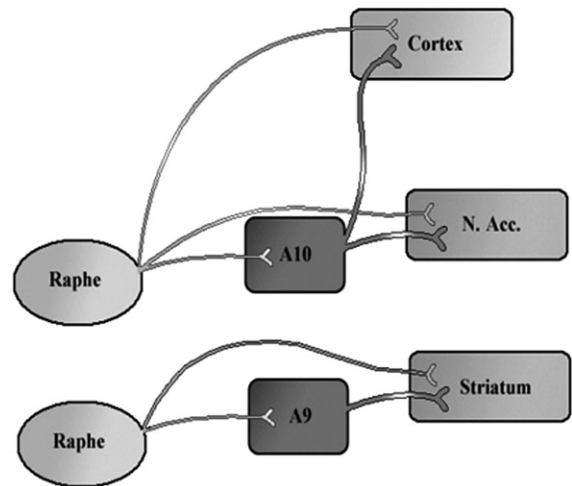


Fig. 1. Schematic representation of serotonin–dopamine interaction in the mesocorticolimbic and nigrostriatal DA-ergic system. Serotonin-containing cell bodies of the raphe nuclei send projections to dopaminergic cells in both the ventral tegmental area (VTA, A10) and the substantia nigra (SN, A9), and to their terminal fields in the nucleus accumbens, prefrontal cortex and striatum.

5-HT receptors localization

The diverse physiological effects of 5-HT in the brain are mediated by a variety of distinct receptors. These receptors are presently divided into 7 classes (5-HT₁–5-HT₇), which are then subdivided into subclasses with a total of at least 14 different receptors (Fig. 2), based on their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms (Hoyer et al., 1994, 2002; Barnes and Sharp, 1999). With the exception of the ionotropic 5-HT₃ receptor, all other 5-HT receptors are G-protein-coupled receptors (metabotropic) and act through intracellular signalling pathways to hyperpolarize, in the case of 5-HT₁ receptors, or depolarize, in the case of the remaining 5-HT receptor classes, their host cells (Barnes and Sharp, 1999).

The 5-HT₁ receptor class

The 5-HT₁ receptor class is comprised of five receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}), which, in humans, share 40–63%

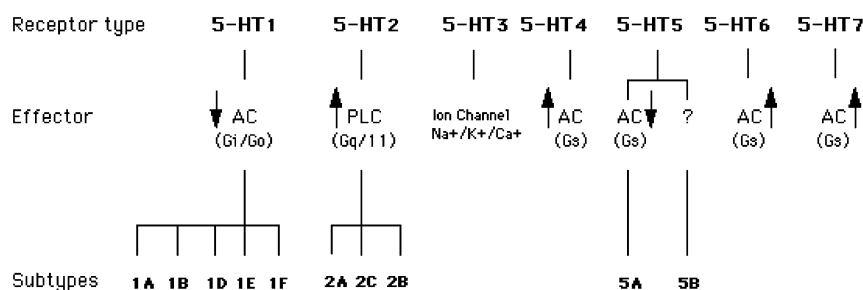


Fig. 2. *Serotonin receptors*: 5-HT receptors are at present divided into seven classes and further on subclasses, for a total of fourteen 5-HT receptors. With the exception of the 5-HT₃ receptor, which forms a ligand-gated ion channel, all 5-HT receptors belong to the superfamily of G-protein-coupled receptors containing seven-transmembrane domain structures.

overall sequence identity and couple preferentially, although not exclusively, to G_{i/o} to inhibit cAMP formation. While for the 5-HT_{1E} and 5-HT_{1F} receptors, physiological roles have not yet been found, 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors have been demonstrated functionally in a variety of tissues from various species (Barnes and Sharp, 1999; Hoyer et al., 2002). Although all 5-HT receptor subtypes are localized postsynaptically on 5-HT target cells, the 5-HT_{1A} and 5-HT_{1B} subtypes are also located presynaptically on 5-HT neurons. In the raphe nuclei, 5-HT_{1A} autoreceptors are localized on 5-HT cell bodies and dendrites where they function as autoreceptors and the 5-HT_{1B} receptors are localized presynaptically on 5-HT nerve terminals where they serve to regulate 5-HT release (Pompeiano et al., 1992; Barnes and Sharp, 1999; Hoyer et al., 2002).

Although 5-HT_{1A} are predominantly somatodendritic receptors, postsynaptically they are widely located in structures of the mesocortico-limbic DA system.

A high 5-HT_{1A} receptors density has been found in the hippocampus, the lateral septum and the amygdala, and in cortical areas, such as the entorhinal and cingulate cortices. In the hippocampus and cortex, 5-HT_{1A} receptors are found in pyramidal cells. Medium binding was detected in the olfactory bulb, the thalamus, hypothalamus, several brain stem nuclei and the neocortex. Low levels, or no binding, were detected in the basal ganglia and cerebellum (Pompeiano et al., 1992). In the prefrontal cortex, they are highly co-localized with 5-HT_{2A} receptors, and 5-HT_{1A}

mRNA is also observed in cortical GABA-ergic cells (Amargós-Bosch et al., 2004; Santana et al., 2004). Moreover, 5-HT_{1A} receptor protein was also found in the VTA, mainly expressed in the parabrachial subdivision of the VTA which projects preferentially to the prefrontal cortex (Doherty and Pickel, 2001) and not only exclusively localized on DA-ergic neurons, but also on non-DA-ergic neurons and glia (Doherty and Pickel, 2001).

The 5-HT_{1B} receptors have been identified by radioligand binding techniques, predominantly in the basal ganglia of the rat and mouse brain, particularly in the SN, globus pallidus, caudate putamen, ventral pallidum and entopeduncular nucleus, and also in many other regions such as the hippocampus, cortex and VTA (Bruinvels et al., 1993) where a large proportion of these are probably located on the terminal of GABA-ergic cells (Bruinvels et al., 1994). As already mentioned, data from many studies suggest that 5-HT_{1B} receptors are located on terminals presynaptically and postsynaptically relative to the 5-HT neurons where they play the role of both 5-HT autoreceptors and 5-HT heteroreceptors. The former control the release of 5-HT, while the latter control the release of non-5-HT neurotransmitters (Boschert et al., 1994; Morikawa et al., 2000).

5-HT_{1D} receptors seem to be co-localized with 5-HT_{1B} receptors, although at much lower densities (Bruinvels et al., 1993) and a distinct pharmacological profile.

The mRNA for the 5-HT_{1E} receptor subtype is present in dopaminergic brain regions including

the caudate, putamen and amygdala as well as cortical areas (Bruinvels et al., 1994); likewise, the mRNA for the 5-HT_{1F} receptor subtype has been detected in the hippocampus as well as in cortical areas (Bruinvels et al., 1994). However, to date, there is no evidence for a role of the 5-HT_{1D}, 5-HT_{1E} or 5-HT_{1F} receptors in the modulation of dopaminergic activity.

The 5-HT₂ receptor family

5-HT₂ receptors form a closely related subgroup of G-protein-coupled receptors, functionally linked to the phosphatidylinositol hydrolysis pathway and currently classified as 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes (Boess and Martin, 1994; Hoyer et al., 1994; Barnes and Sharp, 1999), based on their close structural homology and pharmacology (Boess and Martin, 1994; Hoyer et al., 1994; Barnes and Sharp, 1999). There is a high-sequence homology (>80% in the transmembrane regions) between the mouse, rat and human 5-HT_{2C} receptors (Barnes and Sharp, 1999), and it is not surprising that many compounds bind with high affinity all these three receptor subtypes.

5-HT_{2C} receptors are widely distributed throughout the brain and have been proposed as the main mediators of the different actions of 5-HT in the CNS (Boess and Martin, 1994; Hoyer et al., 1994; Barnes and Sharp, 1999). High levels of 5-HT_{2C} mRNA or protein expression have been found in the choroid plexus, the frontal cortex, limbic structures such as hippocampus, septum and hypothalamus, and also in the striatum, nucleus accumbens, rhombencephalon and spinal cord. The presence of these receptors has also been demonstrated on DA and non-DA cells in the VTA, SNc and the SNr (Grace and Bunney, 1985; Molineaux et al., 1989; Pompeiano et al., 1994; Wright et al., 1995; Sharma et al., 1997; Clemett et al., 2000; Bubar and Cunningham, 2007). The regional and cellular distribution of 5-HT_{2C} receptors was also investigated in the human brain. The main sites of mRNA 5-HT_{2C} receptors or protein expression were the choroid plexus, cerebral cortex, hippocampus, amygdala, some components of the basal ganglia and other limbic structures (Abramowski et al., 1995; Pasqualetti et al., 1999),

suggesting that this receptor might be involved in the regulation of different human brain functions, and might play a role in the pathophysiology of several mental disorders (Kennett, 1993; Baxter et al., 1995; Jenck et al., 1998; Di Matteo et al., 2001; Higgins and Fletcher, 2003; Giorgetti and Tecott, 2004; Alex and Pehek, 2007). There is now evidence that the 5-HT_{2C} receptor is mainly located postsynaptically within dopaminergic, GABA-ergic, cholinergic, substance P, dynorphin and other systems (Ward and Dorsa, 1996; Eberle-Wang et al., 1997; Barnes and Sharp, 1999; Bubar and Cunningham, 2007). Interestingly, the studies by Eberle-Wang et al. (1997) showed the presence of 5-HT_{2C} mRNA within inhibitory GABA-ergic interneurons making direct synaptic contact with SNc and VTA dopaminergic cell bodies. Other immunohistochemical and electrophysiological studies demonstrated an important role of 5-HT_{2C} receptors, localized on non-DA neurons, presumably GABA-ergic, in the regulation of DA cells in the VTA (Van Bockstaele and Pickel, 1995; Steffensen et al., 1998; Bubar and Cunningham, 2007), in the mPFC (Liu et al., 2007) as well as in the SNc (Di Giovanni et al., 2001; Invernizzi et al., 2007) (Fig. 3).

Recent studies found a somatodendritic localization of 5-HT_{2A} receptors on DA neurons in

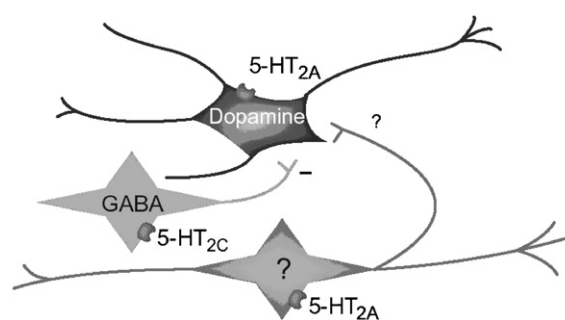


Fig. 3. Distribution of 5-HT_{2A} and 5-HT_{2C} receptors on GABA- and DA-containing neurons in the midbrain. 5-HT_{2A} receptors are expressed on a subpopulation of DA-containing neurons, and on non-DA neurons whose neurochemical identity is as yet unknown (indicated by the question mark). 5-HT_{2C} receptors are expressed on GABA-containing neurons in both the substantia nigra pars reticulata (SNr) and the ventral tegmental area (VTA).

both the parabrachial and the paranigral subdivisions of the VTA (Doherty and Pickel, 2000; Nocjar et al., 2002), which project mainly to the prefrontal cortex and nucleus accumbens, respectively. In addition, 5-HT_{2A} immunoreactivity was also expressed on non-DA cells in the VTA, providing a potential anatomical basis for the modulation of DA neurons in the VTA either directly, by 5-HT_{2A} receptors localized on DA cell, or indirectly, through receptors present on non-DA (presumably GABA-ergic) neurons (Doherty and Pickel, 2000; Nocjar et al., 2002). These receptors were also found at high concentrations in various cortical regions (Wright et al., 1995; Doherty and Pickel, 2000). It is likely that 5-HT_{2A} receptors could affect DA function by acting at the level of dopaminergic nerve terminals, although no direct evidence for the presence of 5-HT_{2A} receptors on such terminals has been provided so far.

Using sensitive techniques, several groups have also shown the presence of both 5-HT_{2B} receptor mRNA (Flanigan et al., 1995) and protein (Duxon et al., 1997) in the rat brain, including midbrain regions. Although there are regional differences in the distribution of these receptors, they are all expressed in the brain with extensive pharmacological and functional similarities, so that it is often difficult to ascribe particular functions to a receptor subtype.

The 5-HT₃ receptors

Among the receptors for 5-HT, the 5-HT₃ receptor is the only ligand-gated cation ion channel located in the central and peripheral nervous system; it has also been detected on a variety of other cells. In the periphery, it is found on autonomic neurons and on neurons of the sensory and enteric nervous system (Barnes and Sharp, 1999). In the CNS, the highest densities of 5-HT₃-receptor-binding sites have been detected in several nuclei in the caudal medulla, such as the area postrema, the nucleus of the solitary tract, the dorsal motor nucleus of the vagus and the nucleus of the spinal tract of the trigeminal nerve. In addition, 5-HT₃-receptor-binding sites have been found in the forebrain, albeit at lower

densities and with a scattered distribution (Barnes and Sharp, 1999; Hoyer et al., 2002), in dopaminergic brain regions such as the SN, the nucleus accumbens and the prefrontal cortex, and at lower densities in the striatum (Kilpatrick et al., 1987; Laporte et al., 1992; Morales et al., 1998). Furthermore, 5-HT₃ receptors were found mainly expressed on GABA-containing cells in the rat neocortex, olfactory cortex, hippocampus and amygdala, which also often contains cholecystokinin (CCK) immunoreactivity (Morales et al., 1996, 1998; Morales and Bloom, 1997), suggesting the participation of 5-HT₃ receptors in the excitation of inhibitory neurons in these brain regions.

The 5-HT₄ receptors

The 5-HT₄ receptor is a G-protein-coupled, seven-transmembrane domain protein, positively linked to the activation of adenylate cyclase (Hoyer et al., 2002). The highest densities of the 5-HT₄ receptor within the CNS are located in limbic regions related to cognitive functions (Eglen et al., 1995). Abundant expression of the 5-HT₄ receptor mRNA was observed in the olfactory system, striatum, medial habenula and the hippocampal formation (Ullmer et al., 1996; Vilario et al., 1996, 2005), while faint or no specific signals could be detected in most other areas of the brain. Interestingly, following selective lesions of either nigrostriatal DA neurons or cell bodies in the striatum, it was found that 5-HT₄ receptors are not located on DA-ergic neurons, on either their cell bodies in the SN or terminals in the striatum, but are likely on the GABA-ergic projection neurons and possibly on cholinergic and GABA-ergic interneurons (Patel et al., 1995).

The 5-HT₅, 5-HT₆ and 5-HT₇ receptors

The 5-HT₅ receptor family consists of two members designated as 5-HT_{5A} and 5-HT_{5B}. To date, the 5-HT_{5A} receptor has been identified in the mouse, rat and human (Barnes and Sharp, 1999; Hoyer et al., 2002). The 5-HT_{5B} receptor also is expressed in the mouse and rat, but not in the human where the coding sequence is interrupted by stop codons.

Both receptors are essentially limited in distribution to the CNS. The 5-HT_{5A} receptor has been demonstrated to couple to G proteins, through Gi/o to inhibit adenylyl cyclase activity (Nelson, 2004). The 5-HT₅ receptors have not been extensively characterized pharmacologically, and as yet their role in the regulation of DA-ergic central function is not known.

Regional analyses of the expression of 5-HT₆ receptor mRNA have revealed that the highest levels are found in the striatum, olfactory tubercle, nucleus accumbens and subfields of the hippocampus, with lesser expression in the cerebral cortex (Ruat et al., 1993a; Gerard et al., 1996, 1997). It seems likely, therefore, that 5-HT₆ receptors are located on GABA-ergic or other intrinsic neurons, at least in the striatum (Roberts et al., 2002). The dense concentrations of 5-HT₆ receptor binding sites in DA-ergic areas such as the striatum and nucleus accumbens are a common feature of all the studies, suggesting that there could be some modulation of DA neuronal function by 5-HT via 5-HT₆ receptors in these brain regions.

A Northern blot analysis of various mammalian tissues has shown the highest levels of 5-HT₇ mRNA in the hypothalamus and thalamus, with high amounts in the brainstem and hippocampus, and lower levels in the cerebral cortex, striatum, olfactory bulb and olfactory tubercle (Ruat et al., 1993b; Shen et al., 1993). The expression of mRNA for 5-HT₇ receptors in thalamic and limbic structures points to their role in affective behaviour, and the fact that such antipsychotics show a high affinity for 5-HT₆ and 5-HT₇ receptors and have features of antagonists (Roth et al., 1994) has led to an assumption that these receptors may be important for mediating the unique actions of certain antipsychotic drugs (APDs).

Microdialysis

Microdialysis coupled to high-performance liquid chromatography (HPLC) is an established technique for studying physiological, pharmacological and pathological changes of a wide range of low molecular weight substances in the brain extracellular fluid. It is based on the idea that a probe

(Fig. 4) made of a hollow fibre permeable to solutes of low molecular weight inserted into the brain tissue mimics blood capillaries in exchanging material from and to the extracellular fluid (Ungerstedt, 1991). The two main areas of application of microdialysis are the recovery of endogenous substances, primarily the neurotransmitters, and the infusion of drugs through the microdialysis cannula (reverse dialysis). Indeed, microdialysis has been developed during the last 25 years by several authors primarily to study brain function and changes in levels of endogenous compounds such as neurotransmitters or metabolites. Nevertheless, in central nervous studies, reverse microdialysis has been extensively used for the study of the effects on neurotransmission at different central nuclei of diverse pharmacological and toxicological agents, such as antidepressants, antipsychotics, anti-Parkinsonians, hallucinogens, drugs of abuse and experimental drugs. Thus, the microdialysis approach has largely contributed not only to clarification of the physiological role of the serotonergic and dopaminergic neuronal systems but also to the development of therapeutic strategies for the treatment of a number of neuropsychiatric disorders.

5-HT_{1A} receptors and dopamine function

The involvement of 5-HT_{1A} receptors in the control of central DA-ergic function is supported by a vast number of neurochemical studies. Thus, the 5-HT_{1A} receptor agonists have complex effects on DA neurotransmission, in a region-specific manner. The selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) reduced 5-HT levels and increased those of DA in the mPFC and hippocampus of rats, while the selective 5-HT_{1A} antagonist, WAY 100635 [*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide], which had little or no effect on monoamine levels alone, suggesting that 5-HT_{1A} receptors do not have a role in the modulation of tonic DA release in mPFC, abolished the influence of 8-OH-DPAT on 5-HT and DA levels in the same area (Arborelius et al., 1993; Rasmusson et al., 1994;

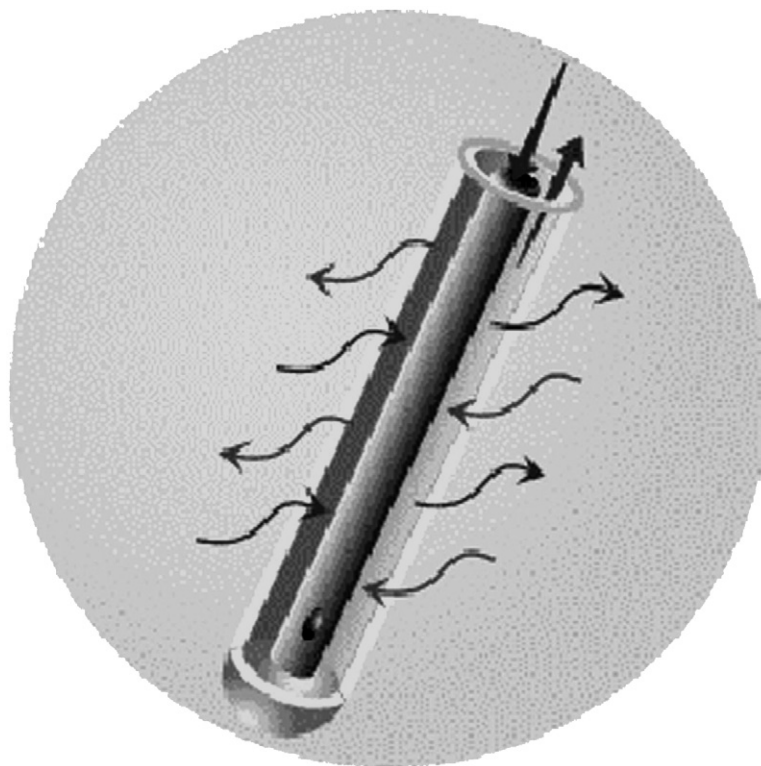


Fig. 4. *The microdialysis probe*: A microdialysis probe is usually constructed as a concentric tube where the perfusion fluid enters through an inner tube, flows to its distal end, exits the tube and enters the space between the inner tube and the outer dialysis membrane. The direction of flow is now reversed and the fluid moves towards the proximal end of the probe. This is where the 'dialysis' takes place, i.e. the diffusion of molecules between the extracellular fluid and the perfusion fluid.

Tanda et al., 1994; Gobert et al., 1998; Rollema et al., 2000; Ichikawa et al., 2001b; Assié et al., 2005). A different situation emerged when stimulation of 5-HT_{1A} receptors had no effects, or decreased accumbal and striatal DA release (Arborelius et al., 1993; Rasmusson et al., 1994; Tanda et al., 1994; Ichikawa and Meltzer, 1999a, 2000; Rollema et al., 2000). In line with these findings, 5-{3-[(2*S*)-1,4-benzodioxan-2-ylmethyl]amino]propoxy}-1,3-benzodioxole (MKC-242), another potent and selective 5-HT_{1A} receptor agonist with anxiolytic and antidepressant-like effects, increased DA release in the prefrontal cortex and hippocampus, but not in the striatum or nucleus accumbens. Furthermore, the 5-HT_{1A} receptor agonist-induced increase in DA release was greater in the hippocampus than in the prefrontal cortex. This region-specific effect was

explained by the idea that DA outflow is modulated by postsynaptic 5-HT_{1A} receptors (Sakaue et al., 2000) as these are dense in the hippocampus and mPFC, but are sparse in the striatum and nucleus accumbens (Pompeiano et al., 1992). In addition, treatment with 5,7-DHT that destroys presynaptic serotonergic nerve fibres did not alter the effect of MKC-242 in increasing cortical DA release, suggesting that DA efflux is modulated in part by postsynaptic 5-HT_{1A} receptors in the prefrontal cortex (Sakaue et al., 2000). The involvement of the postsynaptic 5-HT_{1A} receptors in MKC-242-induced cortical DA release is further supported by employing local application of 5-HT_{1A} receptor agonists and antagonists. The effect of MKC-242 to increase cortical DA release was blocked by local application of WAY 100635, and local application of

8-OH-DPAT increased DA release in the cortex. A long duration of WAY 100635 or 8-OH-DPAT perfusion was required, suggesting that the site of action of these drugs was away from the position of the probe: thus, postsynaptic 5-HT_{1A} receptors modulating cortical DA release appeared to be localized on sites other than dopaminergic nerve terminals (Sakaue et al., 2000; Ago et al., 2003).

The fact that the activity of DA-ergic neurons in the VTA and the mesocortical DA release are mainly modulated by postsynaptic 5-HT_{1A} receptors, while 5-HT release is both pre- and post-synaptically controlled, was confirmed by a recent study of Díaz-Mataix et al. (2005) (Fig. 5). Systemic administration of the highly selective 5-HT_{1A} agonist *R*-(-)-2-(4-[(chroman-2-ylmethyl)-amino]-butyl)-1,1-dioxo-benzo[*d*] isothiazolone hydrochloride (BAY) increased the firing rate and bursting activity of DA-ergic neurons in the VTA and enhanced DA release in both VTA and mPFC in rats and mice; these effects were reversed by WAY 10063. Interestingly, BAY did not alter DA cell activity or DA release in the VTA of cortically

transected rats, further suggesting the involvement of mPFC 5-HT_{1A} receptors. On the other hand, local application of BAY produced a biphasic effect on cortical DA release: a low concentration increased DA release, was blocked by bicuculline, a GABA_A receptor antagonist, while a higher concentration decreased DA efflux — both effects appeared to be due to the activation of 5-HT_{1A} receptors. In the first case, low BAY concentrations preferentially activated 5-HT_{1A} receptors located on GABA-ergic interneurons resulting in a disinhibition of pyramidal neurons projecting to the VTA. A higher BAY concentration might overcome this effect, activating directly pyramidal 5-HT_{1A} receptors and reducing the prefrontal excitatory output to DA neurons. Further, these effects were not observed in the 5-HT_{1A} knockout mice (Díaz-Mataix et al., 2005).

Interestingly, the superior clinical efficacy of clozapine, the prototype atypical APD, may be related to its ability to selectively increase DA release in the prefrontal cortex and hippocampus, rather than in the nucleus accumbens and

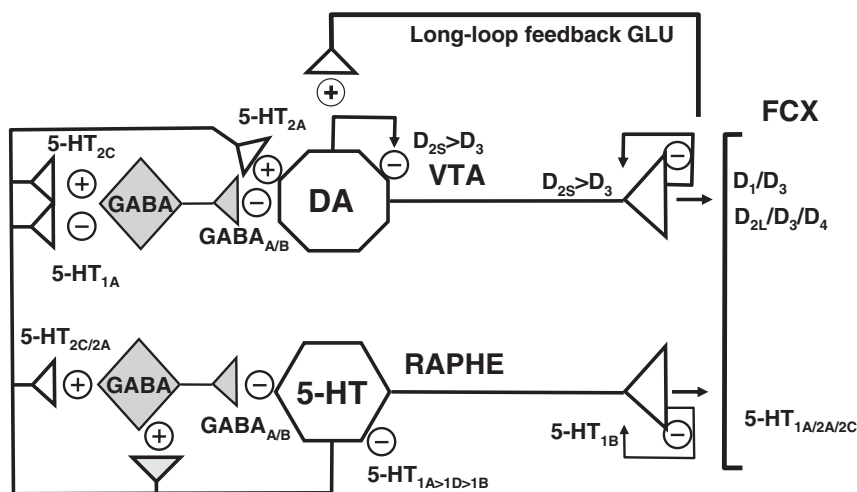


Fig. 5. Schematic representation of the putative modulation of dopaminergic transmission in the frontal cortex (FCX) by diverse classes of serotonergic auto- and heteroreceptors in relation to GABA-ergic interneurons. GABA-ergic interneurons provide a link between several classes of serotonergic receptor and dopaminergic neurons. With regard to 5-HT_{1A} receptors, they directly inhibit the activity of raphe-localized serotonergic cell bodies (autoreceptors). Most importantly, activation of 5-HT_{1A} autoreceptors relieves tonic activity at excitatory 5-HT_{2C} receptors on GABA-ergic interneurons. Stimulation of cortical 5-HT_{1A} receptors, localized on GABA-ergic neurons or 5-HT_{2A} receptors directly on pyramidal neurons, may stimulate DA-ergic cells in the VTA mediated by descending glutamatergic (GLU) pathway. 5-HT_{2C} receptors localized on GABA interneurons, stimulating the release of GABA which inhibits mesocortical DA cells. 5-HT_{2A} receptors stimulate DA neurons directly. This is attenuated by a concomitant stimulatory action of 5-HT_{2A} receptors on GABA interneurons which inhibit DA cells.

striatum. This is in part due to 5-HT_{1A} receptors stimulation, as demonstrated by measuring the decrease in extracellular 5-HT in the rat hippocampus, a region that has been widely used to assess activation of somatodendritic 5-HT_{1A} auto-receptors in the raphe nuclei, and by selective antagonism at 5-HT_{1A} receptors on 5-HT and DA efflux in both areas (Moghaddam and Bunney, 1990; Volonté et al., 1997; Millan et al., 1998b; Kuroki et al., 1999b; Rollema et al., 2000; Ichikawa et al., 2001b; Hagino and Watanabe, 2002; Claustre et al., 2003; Chung et al., 2004; Assié et al., 2005; Díaz-Mataix et al., 2005). Like clozapine, numerous atypical APDs such as amperozide, olanzapine, risperidone, loxapine, ziprasidone, BIMG 80 (Volonté et al., 1997; Kuroki et al., 1999b; Rollema et al., 2000; Ichikawa et al., 2001b; Li et al., 2003; Díaz-Mataix et al., 2005), quetiapine, iloperone, melperone (Ichikawa et al., 2002) and aripiprazole (Li et al., 2004; Zocchi et al., 2005; Bortolozzi et al., 2007) produced greater increase in extracellular DA in the mPFC or hippocampus than in the nucleus accumbens and striatum, attenuated by WAY 10063. Since 8-OH-DPAT potentiated sulpiride-induced increase of DA release in mPFC and nucleus accumbens but not in the striatum (Ichikawa and Meltzer, 1999a), and ritanserin that of raclopride (Andersson et al., 1995), Ichikawa et al. (2001b) demonstrated that the combination of 5-HT_{2A} and D₂ receptor blockade increases DA release in the mPFC, via activation of 5-HT_{1A} receptors, a common feature of most atypical antipsychotics to improve negative symptoms and cognitive dysfunctions in schizophrenia. Interestingly, 8-OH-DPAT, or amperozide, and M100907, two 5HT_{2A} receptor antagonists, inhibited the ability of amphetamine to increase DA release in rat nucleus accumbens and striatum (Ichikawa and Meltzer, 1992; Ichikawa et al., 1995; Liégeois et al., 2002). Thus, attenuation of stimulated DA release in the nucleus accumbens and striatum by 5-HT_{1A} receptor agonism and/or 5-HT_{2A} antagonism may contribute to reverse neuroleptic-induced catalepsy in rats. In this respect, combining antagonist/partial agonist activity at DA D₂ and agonist activity at 5-HT_{1A} receptors is one of the approaches that has recently been chosen to develop the

new generation of antipsychotics, including bifeprunox, SSR181507 and SLV313, in that 5-HT_{1A} receptor activation greatly reduces or prevents the cataleptogenic potential of these novel antipsychotics (Claustre et al., 2003; Assié et al., 2005; McCreary et al., 2007).

Furthermore, serotonergic regulation of the mesocorticolimbic DA-ergic pathway plays an important role in the effects of antidepressants (Tanda et al., 1994). Valproic acid, carbamazepine and zonisamide, three anticonvulsant mood stabilizers, have been reported to preferentially increase DA release in the mPFC of rats, sharing a common mechanism of action mediated by 5-HT_{1A} receptor activation (Ichikawa and Meltzer, 1999b; Ichikawa et al., 2005). Thus, anticonvulsant mood stabilizers, as well as atypical APDs, would be expected to ameliorate or prevent depression, at least in part, via reversal of decreased prefrontal cortical activity by facilitating 5-HT_{1A} activation and its resultant increase in mPFC DA release. Moreover, a number of antidepressant agents, such as flibanserin (Invernizzi et al., 2003), ipsapirone (Wędzony et al., 1996), mirtazapine (Nakayama et al., 2004), fluoxetine and buspirone (Gobert et al., 1999; Sakaue et al., 2000), all drugs showing agonistic properties at 5-HT_{1A} receptors, raised extracellular DA in mPFC, markedly, attenuated by pretreatment with WAY 100635. Also the combination of atypical APDs in addition to 5-HT reuptake inhibitors has recently proven to be beneficial in a number of neuropsychiatric disorders, such as resistant depression, schizophrenia and obsessive-compulsive disorder, as this route markedly potentiates mPFC DA release, compared to that elicited by the administration of a single drug alone. Thus, activation of 5-HT_{1A} receptors secondary to the combined blockade of 5-HT_{2A} and D_{2/3} receptors seems to be relevant for this action (Gobert et al., 1997, 1999; Gobert and Millan, 1999a; Yoshino et al., 2002, 2004; Denys et al., 2004; Ago et al., 2005; Huang et al., 2006; Bortolozzi et al., 2007).

5-HT_{1B} receptors and dopamine function

Neurochemical studies suggest an important role of the 5-HT_{1B} receptor in modulating the activity

of mesoaccumbens and mesostriatal DA-ergic neurons. Evidence that the activation of these receptors facilitates DA neurotransmission has also been obtained. Administration of the 5-HT_{1B} receptor agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxy-pyrrolo[3,2-*b*]pyridine (CP 93129) into the VTA has been shown to increase DA levels in the nucleus accumbens (Yan and Yan, 2001; O'Dell and Parsons, 2004; Yan et al., 2004) and concurrently decrease GABA in the VTA (O'Dell and Parsons, 2004; Yan et al., 2004), both antagonized by co-infusion of *N*-[3-[3-(dimethylamino)ethoxy]-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-carboxamide hydrochloride (SB 216641), a 5-HT_{1B} selective antagonist, but not by WAY 100635, a 5-HT_{1A} antagonist, or 4-[3-chlorophenyl]- α -[diphenylmethyl]-1-piperazineethanol hydrochloride (BRL 15572), a 5-HT_{1D/1A} antagonist (Yan et al., 2004). Systemic 5-HT_{1B} agonism also decreased VTA GABA (Parsons et al., 1999) and increased DA release in the nucleus accumbens (Boulenguez et al., 1996). These studies suggest that 5-HT_{1B} receptors within the VTA regulate mesolimbic DA activity by inhibiting GABA release. Administration of CP 93129, into the nucleus accumbens, also resulted in a local increase of DA release (Yan and Yan, 2001). While stimulation of accumbal 5-HT_{1B} receptors phasically increased DA release, administration of the 5-HT_{1B} antagonist [4-(5-methoxy-3-(4-methyl-piperazin-1-yl))-phenyl] amide (GR 127935) alone into the nucleus accumbens had no effect on basal DA levels (Hållbus et al., 1997), indicating that accumbal 5-HT_{1B} receptors do not tonically modulate mesolimbic DA release.

Increases in DA levels in the dorsal striatum are also observed in response to 5-HT_{1B} receptor stimulation (Benloucif et al., 1993; Galloway et al., 1993; Bentué-Ferrer et al., 1998). These effects have been attributed to an inhibition of GABA release and a disinhibition of DA neuronal activity (Johnson et al., 1992).

Less is known about 5-HT_{1B} receptor regulation of the mesocortical pathway, but some studies have suggested a facilitative role. Local application of 5-HT or a 5-HT_{1B} receptor agonist (CP 93129 or CP 94253) in the mPFC increased cortical DA release, which was blocked by the 5-HT_{1B} receptor

antagonist GR 127935 (Iyer and Bradberry, 1996). In addition, local pretreatment with GR 127935 has been shown to attenuate the increase in mPFC DA release seen in response to intracortical administration of fluoxetine. This result suggests that fluoxetine-induced increases in synaptic 5-HT levels activate 5-HT_{1B} receptors and thereby act to facilitate DA release in the mPFC (Matsumoto et al., 1999).

Pharmacological studies have shown that the acute administration of 5-HT_{1B} receptor agonists augments cocaine-evoked DA overflow within the nucleus accumbens (Parsons et al., 1999). The ability of extracellular 5-HT to facilitate mesolimbic DA release through 5-HT_{1B} receptors has implications for psychostimulant abuse. Likewise, studies have shown that systemic or intra-VTA 5-HT_{1B} receptor agonism (CP 93129 and RU 24969, respectively) potentiated cocaine-induced increase in DA efflux in the nucleus accumbens and decreased GABA release in the VTA (Parsons et al., 1999; O'Dell and Parsons, 2004). Dopaminergic activity from the mesolimbic pathway may then be disinhibited by the stimulation of 5-HT_{1B} receptors on GABA-ergic projection neurons from the nucleus accumbens to the VTA, resulting in a potentiated response to cocaine. There is also evidence that VTA 5-HT_{1B} receptors may be involved, in part, in mediating the activating effects of ethanol on mesolimbic DA neurons, in that activation and blockade of VTA 5-HT_{1B} receptors potentiated and attenuated, respectively, the ethanol-induced increases in extracellular DA concentrations in both the VTA and the ipsilateral nucleus accumbens (Yan et al., 2005). Taken together, these data suggest that 5-HT_{1B} mesoaccumbal receptors are implicated in the effects of these abused drugs, and antagonism at 5-HT_{1B} receptors may have a role in the consequent therapy.

5-HT_{2A} receptors and dopamine function

It has been reported that stimulation of 5-HT_{2A} receptors by (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), a mixed 5-HT_{2A/2C} receptor agonist, increased DA release

in the mPFC (Gobert and Millan, 1999b; Ichikawa et al., 2001a; Pehek et al., 2001, 2006), and in the posterior nucleus accumbens (Bowers et al., 2000), an effect completely abolished by the selective 5-HT_{2A} receptor antagonist *R*-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M100907), confirming that DOI increases DA release in the mPFC primarily via 5-HT_{2A} receptor stimulation. Although blockade of 5-HT_{2A} receptors by itself did not significantly affect basal DA release in the mPFC (Gobert and Millan, 1999b; Kuroki et al., 1999a; Ichikawa et al., 2001b; Pehek et al., 2001, 2006; Bonaccorso et al., 2002), local infusion of the M100907 into the mPFC attenuated K⁺ and DOI-induced DA release, indicating that, under activated conditions, cortical 5-HT_{2A} receptors potentiate the phasic release of DA (Pehek et al., 2001, 2006). Systemic or intracortical administration of DOI also increased DA-ergic activity and glutamate efflux in the VTA (Bortolozzi et al., 2005; Pehek et al., 2006) and infusions of M100907, directly into the mPFC, blocked this increase, suggesting that regulation of mesocortical DA by cortical 5-HT_{2A} receptors may involve a polysynaptic neural circuit from mPFC to the VTA (Fig. 5). Thus, 5-HT_{2A} receptor-mediated stimulation of corticostriatal projections may result in an enhanced glutamate efflux in the VTA, which subsequently stimulates glutamate receptors on VTA mesocortical neurons, increasing DA neuronal activity and consequently DA release in the mPFC. While, in contrast with above-mentioned data, no significant effect of DOI on basal DA release in the cortex, nucleus accumbens and striatum was reported, it potentiated amphetamine or 3,4-methylenedioxymethamphetamine (MDMA)-induced DA release in these three major DA-ergic terminal areas (Gudelsky et al., 1994; Ichikawa and Meltzer, 1995a; Kuroki et al., 2003). Thus, compelling evidence from biochemical and electrophysiological studies indicates that 5-HT_{2A} receptors may modulate either independent impulse flow or dependent release of DA through mechanisms involving regulation of either DA synthesis or DA neuron firing rate (Schmidt et al., 1992; Gudelsky et al., 1994; Schmidt and Fadayel, 1996).

On the other hand, M100907, which by itself did not affect DA release in all three areas (Schmidt et al., 1992, 1994; Bonaccorso et al., 2002; Liégeois et al., 2002), inhibited the ability of amphetamine and MDMA to increase DA release in the nucleus accumbens and striatum (Schmidt et al., 1992, 1994; Gudelsky et al., 1994). M100907 also attenuated the *N*-methyl-D-aspartate (NMDA) receptor channel blocker dizocilpine-induced DA release in the nucleus accumbens but not in the mPFC (Schmidt and Fadayel, 1996). Furthermore, infusion of the 5-HT_{2A/2C} receptor antagonist ritanserin into either the striatum or the ipsilateral SN attenuated MDMA-induced DA release in the striatum. MDMA treatment also decreased GABA release in the striatum and this was blocked by local ritanserin infusions in either brain site, indicating a role for striatal and nigral 5-HT_{2A} receptors in MDMA-evoked nigrostriatal DA release, mediated by GABA-ergic input to the SN (Yamamoto et al., 1995). Systemic administration of the preferential 5-HT_{2A} receptor antagonist {*trans*-4-[(3*Z*)-3-[(2-dimethylaminoethyl)oxyimino]-3-(2-fluorophenyl)propen-1-yl]phenol hemifumarate} SR46349B, and ritanserin attenuated the ability of electrical stimulation of the DR nucleus to increase DA release in the nucleus accumbens (De Deurwaerdère and Spampinato, 1999). SR46349B, devoid of any effect on basal DA release, also blocked amphetamine and haloperidol-induced DA release in both the nucleus accumbens and the striatum (Lucas et al., 2000; Lucas and Spampinato, 2000; Bonaccorso et al., 2002; Porras et al., 2002b; Auclair et al., 2004). Taken together, these results suggest that 5-HT_{2A} receptor antagonism may inhibit the stimulated DA release. Conversely, 5-HT_{2A} receptor antagonism had no influence on the enhancement of DA release induced by morphine in either nucleus accumbens or striatum (Porras et al., 2002b). It was shown that 5-HT_{2A} and 5-HT_{2C} receptors specifically regulate the activation of midbrain DA neurons induced by amphetamine or morphine, respectively (Porras et al., 2002b). This differential contribution may be conditioned by the specific mechanism of the action of the drug considered and/or by the neuronal circuitry involved in its effect on DA neurons. Therefore,

the fact that drugs of abuse stimulate DA release through different cellular mechanisms leads to the possibility that their effect on DA function could be modulated differentially by each of the 5-HT₂ receptor subtypes (Porrás et al., 2002b). Thus, amphetamine-induced DA release, which occurs independently from DA neuron firing rate, and involves increase in DA synthesis (Seiden et al., 1993; Cadoni et al., 1995), could be sensitive to 5-HT_{2A} but not 5-HT_{2C} receptor regulation. Conversely, morphine-stimulated DA release, thought to be a consequence of its excitatory effect on DA neuron firing rate (Di Chiara and North, 1992), could be controlled by 5-HT_{2C} receptors.

Numerous studies have shown that the so-called 'atypical antipsychotics' such as clozapine, amperozide, olanzapine, risperidone and others, compared to the typical antipsychotics haloperidol or (–) sulpiride, stimulate the release of DA more potently in the mPFC and mesocorticolimbic innervated areas, than in the striatum (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Hertel et al., 1996; Volonté et al., 1997; Kuroki et al., 1999b; Ichikawa et al., 2001b; Westerink et al., 2001; Li et al., 2004). This selective action is associated with a lower incidence of extrapyramidal side-effects (EPS), and with a greater ability to improve negative symptoms and cognitive functions in schizophrenia (Meltzer and Nash, 1991; Roth et al., 1992; Meltzer, 1999; Meltzer et al., 2003). Since a common property of these drugs, that distinguishes them from the typical antipsychotics, is their high affinity for the 5-HT_{2A} receptor, it has been suggested that potent 5-HT_{2A} antagonism, in relation to a weaker DA D₂ receptor antagonism, contributes to their beneficial effects. Thus, pretreatment with the selective 5-HT_{2A} antagonist M100907 before administration of the D₂ antagonists haloperidol, sulpiride or raclopride produced an increase in mPFC DA release, which was not observed by these compounds administered alone (Ichikawa et al., 2001b; Westerink et al., 2001; Bonaccorso et al., 2002; Liégeois et al., 2002). Interestingly, M100907 potentiated low but not high dose haloperidol-induced DA release in the mPFC and inhibited that in the nucleus accumbens (Bonaccorso et al., 2002; Liégeois et al., 2002); thus, weak D₂ and

potent 5-HT_{2A} receptor blockade may have an important influence on the preferential increase of mPFC DA release by the atypical antipsychotics and on their clinical effectiveness.

Further, evidence has been provided that this effect may be mediated by actions of released 5-HT interacting with 5-HT_{1A} receptors. In fact, reversal by WAY 100635 of the potentiation of DA release in mPFC induced by selective antagonism at 5-HT_{2A} and D₂ receptors suggested that facilitation of 5-HT_{1A} receptor stimulation is essential to the simultaneous blockade of 5-HT_{2A} and D₂ receptors to increase cortical DA release (Ichikawa et al., 2001b; Bonaccorso et al., 2002). Agents acting at multi-receptor sites appear to be more promising as APDs, and recent data show that blockade of DA receptors and combined antagonism at 5-HT_{2A} as well as 5-HT_{2C} receptors may be involved in the therapeutic effects of novel antipsychotics (Meltzer, 1999; Bonaccorso et al., 2002; Jones and Blackburn, 2002; Meltzer et al., 2003). Earlier studies demonstrated that administration of ritanserin, a mixed 5-HT_{2A/2C} receptor antagonist, increased nigrostriatal and mesocorticolimbic DA efflux (Devaud et al., 1992; Pehek, 1996; Pehek and Bi, 1997). Interestingly, ritanserin has been reported to potentiate the D_{2/3} receptor antagonist raclopride-induced DA release in the mPFC and nucleus accumbens, but not in the striatum (Andersson et al., 1995). Another putative atypical APD SR46349B, that shares both 5-HT_{2A} and 5-HT_{2C} receptor antagonism, increased cortical DA release and potentiated haloperidol-induced DA release in both mPFC and nucleus accumbens, suggesting that 5-HT_{2C} receptor antagonism may also contribute to the potentiation of DA release produced by haloperidol (Bonaccorso et al., 2002). A novel putative atypical antipsychotic ACP-103, inverse agonist at both 5-HT_{2A} and 5-HT_{2C} receptors, increased DA release in the mPFC but not in the nucleus accumbens, and potentiated low dose of haloperidol-induced DA release in the mPFC, while inhibiting that in the nucleus accumbens (Li et al., 2005). Taken together, these data suggest that combined 5-HT_{2A/2C} receptor antagonism may be more advantageous than selective 5-HT_{2A} antagonism alone as an adjunct to D₂ antagonism

to improve cognition and negative symptoms in schizophrenia.

5-HT_{2C} receptors and dopamine function

Several studies have focused on the role of 5-HT_{2C} receptors in the regulation of forebrain DA function and highlighted their potential as a target for improved treatments of neuropsychiatric disorders related to central DA neuron dysfunction (Jenck et al., 1998; Di Matteo et al., 2001; Higgins and Fletcher, 2003; Giorgetti and Tecott, 2004; Di Giovanni et al., 2006a; Alex and Pehek, 2007). The involvement of 5-HT_{2C} receptor subtypes in the control of mesocorticolimbic and nigrostriatal DA neuron activity is now well established, and evidence has been provided that they exert both tonic and phasic modulation of central dopaminergic function (Prisco et al., 1994; Di Matteo et al., 1998, 1999, 2000a, b, c; Millan et al., 1998a; De Deurwaerdère and Spampinato, 1999; Di Giovanni et al., 1999, 2000; Gobert and Millan, 1999b; Gobert et al., 2000; Hutson et al., 2000; Lucas et al., 2000; Lucas and Spampinato, 2000; Blackburn et al., 2002; Porrás et al., 2002b; Pozzi et al., 2002; Alex et al., 2005; De Deurwaerdère et al., 2004; Navailles et al., 2004, 2006b, 2008).

Initially, in our laboratory, it was found that the firing rate of DA neurons in the VTA was reduced by mCPP and trifluoromethylphenylpiperazine (TFMPP), two mixed 5-HT_{1B/2A/2B/2C} receptor agonists (Hoyer et al., 1994), whereas these neurons were stimulated by mesulergine (Prisco et al., 1994). Based on those findings, it was suggested that 5-HT could exert an inhibitory action on DA neurons in the VTA by acting through 5-HT₂ receptors (Prisco et al., 1994). However, these data did not allow us to distinguish the relative contribution of each 5-HT₂ receptor subtype in the control of central DA function. Subsequently, our and other studies clearly indicated a selective involvement of 5-HT_{2C} receptors for the suppressive influence of 5-HT on the activity of mesocorticolimbic and nigrostriatal dopaminergic pathways. In fact, a series of *in vivo* electrophysiological and neurochemical studies showed that 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB 206553),

a selective 5-HT_{2C/2B} receptor inverse agonist (Kennett et al., 1996; De Deurwaerdère et al., 2004), and 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomoyl] indoline (SB 242084), the most potent and selective 5-HT_{2C} receptor antagonist available (Kennett et al., 1997), increased the basal firing rate and the bursting activity of VTA DA neurons, and enhanced DA release in both rat nucleus accumbens and prefrontal cortex (De Deurwaerdère and Spampinato, 1999; Di Giovanni et al., 1999; Di Matteo et al., 1999; Gobert and Millan, 1999b; Gobert et al., 2000). Conversely, systemic administration of (*S*)-2-(chloro-5-fluoro-indo-1-yl)-1-methylethylamine 1:1 C₄ H₄ O₄ (RO 60-0175), a selective 5-HT_{2C} receptor agonist (Martin et al., 1998), had opposite effects (Millan et al., 1998a; Di Giovanni et al., 1999; Di Matteo et al., 1999b, 2000a, b, c; Gobert et al., 2000). SB 206553 and SB 242084 were also found to potentiate pharmacological-induced accumbal DA release (Hutson et al., 2000; Porrás et al., 2002b; Navailles et al., 2004), and stress-stimulated DA outflow in the rat prefrontal cortex (Pozzi et al., 2002), while stimulation of 5-HT_{2C} receptors by RO 60-0175 in the VTA suppressed it (Pozzi et al., 2002), suggesting a role of these receptors on evoked accumbal and mPFC DA release also. On the other hand, 5-HT_{2C} receptor agonists such as mCPP, MK 212 [6-chloro-2-(1-piperazinyl)piperazine] and RO 60-0175 did not significantly affect the activity of SNc DA neurons and the *in vivo* DA release in the striatum (Di Matteo et al., 1999; Di Giovanni et al., 2000). Moreover, the mixed 5HT_{2B/2C} antagonist SB 206553 caused only a slight increase in the basal activity of DA neurons in the nigrostriatal pathway (Di Giovanni et al., 1999), suggesting that the serotonergic system controls both basal and stimulated impulse flow-dependent release of DA preferentially in the mesocorticolimbic system by acting through 5-HT_{2C} receptors.

Consistently, a study carried out in our laboratory has shown that mCPP excites non-DA (presumably GABA-containing) neurons in both the SNr and the VTA by activating 5-HT_{2C} receptors (Di Giovanni et al., 2001) (Fig. 3). One interesting finding of that study was the differential effect exerted by mCPP on subpopulations of SNr neurons. Thus, mCPP caused a marked

excitation of presumed GABA-ergic SNr projection neurons, whereas it did not affect SNr GABA-containing interneurons that exert a direct inhibitory influence on DA neurons in the SN (Di Giovanni et al., 2001). On the other hand, all non-DA neurons in the VTA were equally excited by mCPP. It is tempting to speculate that this differential response to mCPP might be the basis of the preferential inhibitory effect of 5-HT_{2C} agonists on the mesocorticolimbic versus the nigrostriatal DA function. Other *in vivo* electrophysiological and neurochemical studies have confirmed and extended the above-mentioned data, indicating that 5-HT exerts a direct excitatory effect on GABA-ergic neurons in the SNr and VTA by acting on 5-HT_{2C} receptors (Bankson and Yamamoto, 2004; Invernizzi et al., 2007). In fact, about 50% of SNr neurons are excited by the selective 5-HT_{2C} receptors agonist RO 60-0175 and this effect is counteracted by the new and selective 5-HT_{2C} inverse agonist SB 243213 (5-methyl-1-[(2-methyl-3-pyridyl)oxy]-5-pyridyl carbamoyl]-6-trifluoromethylindoline hydrochloride) (Wood et al., 2001; Berg et al., 2006). In addition, microiontophoretic application of RO 60-0175 clearly showed a direct effect of the 5-HT_{2C} receptors on the SNr neurons, antagonized by SB 243213. Infusion of RO 60-0175 and mCPP by reverse dialysis significantly increased extracellular levels of GABA in the SNr (Invernizzi et al., 2007). Nevertheless, intra-VTA infusion of SB 206553 has been shown to attenuate MDMA-induced increase GABA levels in the VTA and to potentiate the concurrent increase in accumbal DA release (Bankson and Yamamoto, 2004).

Although recent studies showed that systemic administration of 5-HT_{2C} receptor agonists, including RO-600175, does not significantly decrease the activity of nigrostriatal DA-ergic neurons (Di Matteo et al., 1999; Di Giovanni et al., 2000), such treatment decreases DA efflux in the striatum (Gobert et al., 2000; Navailles et al., 2004; Alex et al., 2005), while systemic administration of SB 206553 and SB 242084 enhances it (De Deurwaerdère and Spampinato, 1999; Di Giovanni et al., 1999; Porras et al., 2002b; Navailles et al., 2004). A recent study has shown

that the 5-HT_{2C} receptor inverse agonist-induced increase in accumbal and striatal DA release is insensitive to the depletion of extracellular 5-HT, suggesting that constitutive activity of the 5-HT_{2C} receptors participates in the tonic inhibitory control that they exert on DA release in both the nucleus accumbens and the striatum (De Deurwaerdère et al., 2004). Furthermore, biochemical evidence indicates that both VTA and accumbal 5-HT_{2C} receptors participate in the phasic inhibitory control exerted by central 5-HT_{2C} receptors on mesoaccumbens DA neurons (Navailles et al., 2006b, 2008), and that the nucleus accumbens shell region constitutes the major site for the expression of the tonic inhibitory control involving the constitutive activity of 5-HT_{2C} receptors (Navailles et al., 2006b). There is also evidence that 5-HT_{2C} receptors can modulate the phasic activity of the DA-ergic nigrostriatal system. Indeed, SB 206553 has been shown to potentiate cocaine-, morphine- and haloperidol-induced increase in DA outflow in the rat striatum (Porras et al., 2002b; Navailles et al., 2004, 2006a) and systemic administration of RO 60-0175 was found to attenuate haloperidol-induced DA release in the same area (Navailles et al., 2004), as well as nicotine-induced increase in DA activity in the nigrostriatal system (Di Matteo et al., 2004; Pierucci et al., 2004).

5-HT₃ receptors and dopamine function

Intracortical administration of the 5-HT₃ agonist 1-phenylbiguanide or *n*-methylquipazine (NMQ) has been shown to increase mPFC DA release (Chen et al., 1992; Kurata et al., 1996), suggesting a facilitative role of 5-HT₃ cortical receptors on DA release. Local 1-phenylbiguanide or *m*-chlorophenylbiguanide (mCPBG) and systemic administration of the 5-HT₃ agonist 2-methylserotonin (2-Me-5HT) increased DA release in the nucleus accumbens, also, and this effect, dependent on the impulse flow of DA-ergic cells (Jiang et al., 1990), was blocked by locally applied BRL-43694 (granisetron), zacopride or GR38032F, all selective 5-HT₃ antagonists (Jiang et al., 1990; Chen et al., 1991; Campbell and McBride, 1995). Further, this

effect was also observed in 5-HT-depleted rats, suggesting that 5-HT₃ receptors are located pre-synaptically on DA terminals in the nucleus accumbens (Chen et al., 1991). On the other hand, a role for VTA 5-HT₃ receptors in facilitating somatodendritic DA release in the same area has also been described (Campbell et al., 1996). Interestingly, 5-HT₃ antagonism by systemic ondansetron or (*S*)-zacopride, without affecting basal DA efflux (Carboni et al., 1989; Pei et al., 1993; De Deurwaerdère et al., 1998), significantly attenuated DR nucleus-stimulated DA release in the nucleus accumbens (De Deurwaerdère et al., 1998), suggesting that endogenous 5-HT, via 5-HT₃ receptors, may exert a facilitatory role on accumbal DA outflow. In line with this finding, the elevation of DA release induced by the selective 5-HT uptake inhibitors fluoxetine or mazindol was attenuated by selective 5-HT₃ antagonism in the mPFC (Tanda et al., 1995) and in the nucleus accumbens (Kankaanpää et al., 2002), respectively. Within the nigrostriatal system, intrastriatal 5-HT₃ antagonism by 3-tropanyl-indole-3-carboxylate (ICS205930), MDL72222 or ondansetron, without effect on basal DA efflux, attenuated 5-HT or morphine-induced striatal DA release, suggesting that 5-HT acts at 5-HT₃ receptors to facilitate DA release also in the striatum (Benloucif et al., 1993; Porrás et al., 2003). Furthermore, systemic treatment with a variety of 5-HT₃ antagonists did not affect DA efflux in the striatum, suggesting that 5-HT₃ receptors do not regulate tonic DA levels (Invernizzi et al., 1995; Porrás et al., 2003), but appeared to regulate evoked nigrostriatal DA release. Indeed, systemic administration of 5-HT₃ receptor antagonists ICS205930, ondansetron or MDL72222 attenuated striatal DA release induced by morphine (Porrás et al., 2003) and ethanol (Wozniak et al., 1990), but not by haloperidol, amphetamine or cocaine (Porrás et al., 2003). On the contrary, when haloperidol was co-administered with citalopram, known to elevate 5-HT tone, the resulting increase in DA release was attenuated by 5-HT₃ receptor blockade (Porrás et al., 2003). On the basis of these results, and knowing that enhanced DA release induced by cocaine and amphetamine is due to blocking or inverting the function of the

DA transporter, respectively, while increased DA release by haloperidol or morphine is thought to be a consequence of an increase in DA neuron firing rate, it was suggested that 5-HT₃ receptors modulate nigrostriatal (Porrás et al., 2003) or mesolimbic (Carboni et al., 1989; Cervo et al., 1996; Kankaanpää et al., 2002; De Deurwaerdère et al., 2005) DA function only when the stimulated DA release is depolarization-dependent and both DA and 5-HT tones are concomitantly elevated.

Significantly, 5-HT₃ antagonism has been shown to counteract the increase of accumbal DA release induced by various drugs of abuse such as ethanol, nicotine, morphine or cocaine (Carboni et al., 1989; McNeish et al., 1993; Pei et al., 1993; Kankaanpää et al., 1996, 2002; De Deurwaerdère et al., 2005). Systemically, ICS205-930, ondansetron and MDL72222 attenuated morphine-induced DA release in the nucleus accumbens (Carboni et al., 1989; Imperato and Angelucci, 1989; Pei et al., 1993; De Deurwaerdère et al., 2005); nevertheless, intra-VTA, but not intra-accumbal, infusion of ICS205-930 was able to counteract the action of morphine (Imperato and Angelucci, 1989), suggesting that selective antagonism on VTA 5-HT₃ receptors is able to modulate the morphine's action in the mesolimbic system. Furthermore, intra-accumbal infusion of ondansetron strongly reduced the enhancement of DA release elicited by a high but not a low dose of morphine in the same area (De Deurwaerdère et al., 2005), so, it was proposed that in addition to increased DA tone, increased 5-HT release is required to trigger the excitatory action of accumbal 5-HT₃ receptors on DA release.

Systemic pretreatment with ICS205-930 also attenuated ethanol-induced increase of DA efflux in the nucleus accumbens (Carboni et al., 1989; Wozniak et al., 1990). Furthermore, local infusion of ICS205-930 into the VTA (Campbell et al., 1996) or in the nucleus accumbens (Campbell and McBride, 1995) prevented ethanol's action in both areas. In addition, the 5-HT₃ agonist mCPBG had additive effect on DA release when infused in the nucleus accumbens concomitantly to the systemic injection of ethanol in rats (Campbell and McBride, 1995). Therefore, Yoshimoto et al. (1996) showed that chronic alcohol intake

increases the sensitivity of accumbal 5-HT₃ receptors in rats, thus suggesting their involvement in alcohol dependence.

There are, however, contrasting results in the case of amphetamine or cocaine. Systemic administration of the 5-HT₃ antagonists MDL72222 and zacopride has been shown to attenuate both cocaine- and amphetamine-induced DA release in the nucleus accumbens (McNeish et al., 1993; Kankaanpää et al., 1996, 2002), while other studies have shown that systemic 5-HT₃ receptor antagonism had no effect on DA efflux enhanced by these drugs in the same area (Carboni et al., 1989; Cervo et al., 1996; De Deurwaerdère et al., 2005). Therefore, the fact that drugs of abuse stimulate DA release through different cellular mechanisms leads to the possibility that their effect on DA function could be modulated by the 5-HT₃ receptor only under specific conditions, in that it requires a concomitant increase in both endogenous DA and 5-HT tones and operates selectively on the depolarization-dependent exocytosis of DA.

5-HT₄, 5-HT₅, 5-HT₆ and 5-HT₇ receptors and dopamine function

The majority of studies on 5-HT₄ receptor control of DA focus on the nigrostriatal DA system. Striatal DA release has been shown to be unaltered after systemic administration of preferential 5-HT₄ agonists (Taylor and Routledge, 1996), but increased after local perfusion of 5-HT or 5-HT₄ agonists by reverse dialysis (Benloucif et al., 1993; Bonhomme et al., 1995; Steward et al., 1996; De Deurwaerdère et al., 1997). Conversely, selective 5-HT₄ antagonists, administered systemically (Lucas et al., 2001; Porrás et al., 2002a) and locally, into the striatum (Bonhomme et al., 1995; Pozzi et al., 1995; De Deurwaerdère et al., 1997; Lucas et al., 2001) or in the SN (Thorré et al., 1998), had no effect under basal conditions, indicating that 5-HT₄ receptors do not tonically modulate nigrostriatal DA, but reduce DA release under conditions of increased 5-HT and DA output. Indeed, co-perfusion in the SN of 5-HT and the 5-HT₄ antagonist RS 39604 blocked 5-HT-induced nigral DA efflux (Thorré et al., 1998), and

injection of GR-113808, another 5-HT₄ blocker, into the nigra attenuated the enhancement of striatal DA release induced by morphine (Pozzi et al., 1995). Such a state-dependent control exerted by 5-HT₄ receptors seemed to be restricted to the nigrostriatal DA pathway, in that morphine-stimulated accumbal DA output was insensitive to 5-HT₄ antagonism (Pozzi et al., 1995). Further, the increase in striatal but not accumbal DA release induced by morphine was potentiated by the 5-HT₄ receptor agonist prucalopride and reduced by either GR 125487 or SB 204070, two selective 5-HT₄ receptor antagonists (Porrás et al., 2002a), and GR 125487 attenuated the increase in both striatal DA release and nigral DA neuron firing induced by haloperidol, a compound known to elicit an impulse flow-dependent release of DA (Lucas et al., 2001). Interestingly, 5-HT₄ agents did not modulate the increase in striatal DA release induced by cocaine or amphetamine (Porrás et al., 2002a). Therefore, these findings indicated that enhanced DA neuron activity, although necessary, is not sufficient per se to trigger the 5-HT₄ receptor-mediated control. Further, these data suggest that the ability of 5-HT₄ receptors to control DA neuron activity might be dependent on the specific mechanism of action of a given drug to activate DA neurons, and, ultimately, on the means of DA release (i.e. exocytotic versus non-exocytotic). Indeed, whereas morphine and cocaine elicit an exocytotic release of DA consequent to their action on DA neuron firing rate and DA reuptake sites, respectively, amphetamine elicits a non-exocytotic release of DA which occurs independently from DA neuron impulse flow and involves an increase in DA synthesis (Porrás et al., 2002a). Thus, 5-HT₄ receptors selectively modulate striatal but not accumbal DA exocytosis associated with an increased DA neuron firing rate.

The 5-HT₅ receptor family consists of two members designated as 5-HT_{5A} and 5-HT_{5B}. Although both receptors are sparsely distributed in the CNS of mouse, rat and human, they have not been extensively characterized pharmacologically and as yet their role in the regulation of DA-ergic central function is not known.

Interest for the 5-HT₆ receptor has been based, in part, on the finding that some atypical APDs, as

well as some antidepressant agents, are relatively potent 5-HT₆ receptor antagonists (Monsma et al., 1993; Roth et al., 1994). However, there is considerable confusion over the extent to which 5-HT₆ inhibition affects DA transmission, especially cortical DA release. Recently published studies demonstrated that systemically administration of selective 5-HT₆ receptor antagonists increased dialysate levels of DA, but not 5-HT in the rat prelimbic/infralimbic subregion of the prefrontal cortex (Lacroix et al., 2004) and hippocampus (Li et al., 2007). On the contrary, other studies failed to find a direct effect of 5-HT₆ antagonism on cortical (Dawson et al., 2000, 2001; Dawson and Li, 2003; Li et al., 2007) and hippocampal DA efflux (Dawson et al., 2001). Also, in other DA-ergic regions, such as nucleus accumbens and striatum, the selective blockade of 5-HT₆ receptors had no effect on basal DA release (Dawson et al., 2000, 2001, 2003).

Rather, it appears that 5-HT₆ blockade serves to potentiate DA transmission from stimulatory drugs such as amphetamine (Frantz et al., 2002). For instance, DA-induced outflow by peripherally administered amphetamine was enhanced by 5-HT₆ receptor antagonism, in both the cortex, nucleus accumbens (Frantz et al., 2002) and striatum (Dawson et al., 2003), although more robustly in the frontal cortex. The 5-HT₆ receptor antagonist SB-399885 alone increased DA efflux in the hippocampus but not in the mPFC (Li et al., 2007). It potentiated cortical or hippocampal DA efflux produced by haloperidol and risperidone, suggesting that combined blockade of 5-HT₆, in addition to that of 5-HT_{2A} and D₂ receptors, stimulates DA efflux in the mPFC, whereas combined blockade of 5-HT₆ and D₂ receptors is sufficient to enhance DA release in the hippocampus (Li et al., 2007), supporting a possible therapeutic role for 5-HT₆ receptor antagonists in the treatment of cognitive dysfunctions in schizophrenia.

It is difficult to identify any physiological role for 5-HT₇ receptors in the modulation of central DA function because of a lack of selective ligands. On the other hand, the fact that several APDs have high affinity for these receptors (Roth et al., 1994) has led to the hypothesis that occupancy of 5-HT₇

receptors could contribute to certain therapeutic actions of several atypical antipsychotics. Thus, in a combined neurochemical and behavioural study, Takeda et al. (2005) found decreases in exploration were correlated with decreases in DA and 5-HT turnover in the amygdala, after systemic 5-HT₇ receptors inhibition in mice, suggesting a role of these receptors in the modulation of certain aspects of emotionality.

Serotonin–dopamine interaction and neuropsychiatric disorders

Depression

Although DA has received little attention in biological research on depression, as compared to other monoamines such as 5-HT and noradrenaline, current research on the dopaminergic system is about to change this situation. It is now well established that disturbances of mesolimbic and nigrostriatal DA function are involved in the pathophysiology of depression (Brown and Gershon, 1993; Fibiger, 1995; D'Aquila et al., 2000). Moreover, stress promotes profound and complex alterations involving DA release, metabolism and receptor densities in the mesolimbic system (Puglisi-Allegra et al., 1991; Cabib and Puglisi-Allegra, 1996). It seems that exposure to unavoidable/uncontrollable aversive experiences leads to inhibition of DA release in the mesoaccumbens DA system as well as impaired responding to rewarding and aversive stimuli. These alterations could elicit stress-induced expression and exacerbation of some depressive symptoms in humans (Cabib and Puglisi-Allegra, 1996). Thus, in view of the hypothesis that disinhibition of the mesocorticolimbic DA system underlies the mechanism of action of several antidepressant drugs (Cervo and Samanin, 1987, 1988; Cervo et al., 1990; D'Aquila et al., 2000; Di Matteo et al., 2000b, c), the disinhibitory effect of SB 206553 and SB 242084 on the mesolimbic DA system might open new possibilities for the employment of 5-HT_{2C} receptor antagonists as antidepressants (Di Matteo et al., 1998, 1999, 2000b, c, 2001). This hypothesis is consistent with the suggestion that 5-HT_{2C} receptor blockers might exert antidepressant

activity (Baxter et al., 1995; Jenck et al., 1998; Di Matteo et al., 2000b, c, 2001; Giorgetti and Tecott, 2004). In this respect, it is interesting to note that several antidepressant drugs have been shown to bind with submicromolar affinity to 5-HT_{2C} receptors in the pig brain and to antagonize mCPP-induced penile erections in rats, an effect mediated through the stimulation of central 5-HT_{2C} receptors (Jenck et al., 1993, 1994, 1998). Based on those findings, Di Matteo et al. (2000c) have carried out experiments showing that acute administration of amitriptyline and mianserin, two antidepressants with high affinity for 5-HT_{2C} receptors, enhances DA release in the rat nucleus accumbens by blocking these receptor subtypes, in addition to their other pharmacological properties. Interestingly, amitriptyline and mianserin have been tested in the chronic mild stress-induced anhedonia model of depression and were found to be effective in reversing the stress effects (Sampson et al., 1991; Moreau et al., 1994). The antianhedonic effects of tricyclic antidepressants, mianserin and fluoxetine, were blocked by pretreatment with D₂/D₃ receptor antagonists, thus indicating an involvement of DA in the antidepressant effect of various drugs in this model (Sampson et al., 1991; Willner, 1995). The ability of antidepressants, such as tricyclics, selective 5-HT reuptake inhibitors (SSRIs) and mianserin, to affect DA systems, via indirect mechanisms, was also reported by studies of Tanda et al. (1994, 1996) suggesting that potentiation of DA release in the rat cortex may play a role in the therapeutic action of antidepressants. The chronic mild stress procedure, which induces a depression-like state in animals, was shown to enhance 5-HT_{2C} receptor-mediated function, as measured in vivo by mCPP-induced penile erections. In contrast, two different antidepressant treatments (72-h REM sleep deprivation and 10-day administration of moclobemide, a reversible inhibitor of monoamine oxidase type A) resulted in a reduction of this 5-HT_{2C} receptor-mediated function (Moreau et al., 1993). This was interpreted as an indication that the 5-HT_{2C} receptor may be altered, and presumably may exist in a dysregulated (hypersensitive) state in depressive illness. Thus, adaptive processes resulting from chronic antidepressant treatment (i.e. desensitization and/or down-regulation of 5-HT_{2C} receptors) may play an

important role in reversing the 5-HT_{2C} receptor system supersensitivity resulting from a depressive state (Moreau et al., 1996; Jenck et al., 1998).

In contrast to most other receptors, 5-HT_{2C} is not classically regulated. Indeed, 5-HT_{2C} receptors appear to decrease their responsiveness not only on chronic agonist stimulation, but also, paradoxically, after chronic treatment with antagonists (Van Oekelen et al., 2003; Serretti et al., 2004). This mechanism appears to be related to an internalization process that removes activated cell surface receptors from the plasma membrane involving a phosphorylation step and possible degradation in lysosomes (Van Oekelen et al., 2003). As a large number of psychotropic drugs, including atypical antipsychotics, antidepressants and anxiolytics, can all induce down-regulation of 5-HT_{2C} receptors, it has been suggested that this receptor adaptation plays a role in the therapeutic action of these drugs (Van Oekelen et al., 2003; Serretti et al., 2004).

In this respect, it is interesting to note that chronic treatment with 5-HT₂ agonists or antagonists resulted in a paradoxical down-regulation at the 5-HT_{2A} and 5-HT_{2C} receptors (Barker and Sanders-Bush, 1993; Pranzatelli et al., 1993; Newton and Elliott, 1997; Van Oekelen et al., 2003; Serretti et al., 2004) and it seems that the down-regulation state occurring after chronic exposure to mianserin in isolated systems as well as in cell cultures is a direct receptor-mediated mechanism of this drug at these receptors (Newton and Elliott, 1997). Therefore, the down-regulating capacity of 5-HT_{2C} agonists and antagonists may play a particularly important role in treating the supersensitivity of 5-HT_{2C} receptors resulting from a depressive state (Moreau et al., 1996; Jenck et al., 1998; Serretti et al., 2004).

The possible involvement of 5-HT_{2C} receptors in the pathogenesis of depressive disorders and in the mode of action of antidepressants is further substantiated by several other observations. For example, acute administration of fluoxetine caused a dose-dependent inhibition of the firing rate of VTA DA neurons (Prisco and Esposito, 1995), and decreased DA release in both the nucleus accumbens and the striatum (Ichikawa and

Meltzer, 1995b), but it did not affect the activity of DA cells in the SNc (Prisco and Esposito, 1995). A similar effect, though less pronounced, has been observed with citalopram (Prisco and Esposito, 1995). Furthermore, mesulergine, an unselective 5-HT_{2C} receptor antagonist (Boess and Martin, 1994), as well as the lesion of 5-HT neurons by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), prevented fluoxetine-induced inhibition of VTA DA cells (Prisco and Esposito, 1995). These results indicate that fluoxetine inhibits the mesolimbic DA pathway by enhancing the extracellular level of 5-HT, which would act through 5-HT_{2C} receptors (Prisco and Esposito, 1995). This study also demonstrated that fluoxetine-induced inhibition of DA neurons in the VTA was no longer observed after chronic treatment (21 days) with this drug. Interestingly, mCPP inhibited the firing activity of VTA DA neurons in control animals but not in those chronically treated with fluoxetine (Prisco and Esposito, 1995). The authors suggested that 5-HT_{2C} receptors might be down-regulated after repeated fluoxetine administration. Consistent with this hypothesis is the evidence that chronic treatment with sertraline and citalopram, two SSRIs, induced tolerance to the hypolocomotor effect of mCPP (Maj and Moryl, 1992). This hyposensitivity of 5-HT_{2C} receptors might be a key step for the achievement of an antidepressant effect. Indeed, it is possible to argue that the acute inhibitory effect of fluoxetine on the mesolimbic DA system would mask its clinical efficacy in the early stage of treatment. This masking effect would disappear when the hyposensitivity of 5-HT_{2C} receptors occurs. A series of studies carried out in our laboratory have shown that acute administration of SSRIs such as paroxetine, sertraline and fluvoxamine causes a slight but significant decrease in the basal firing rate of VTA DA neurons (Di Mascio et al., 1998). Therefore, it is conceivable that, similar to fluoxetine, these SSRIs could reduce mesocorticolimbic DA transmission by activating 5-HT_{2C} receptors. Furthermore, employing complementary electrophysiological and neurochemical approaches, and both acute and chronic administration routes, it was found that mirtazapine, nefazodone and agomelatine, three effective and innovative

antidepressants, elicit a robust and pronounced enhancement in the activity of mesocorticolimbic DA pathways. These actions were ascribed to their antagonistic properties at inhibitory, tonically active 5-HT_{2C} receptors, that desensitize after repeated drug administration (Millan et al., 2000, 2003; Dremencov et al., 2005).

Interestingly, agomelatine has shown antidepressant efficacy in clinical trials (Lôo et al., 2002; Pandi-Perumal et al., 2006; Zupancic and Guilleminault, 2006), and, indeed, it was found to be effective in treating severe depression associated with anxiety symptoms, with a better tolerability and lower adverse effects than other antidepressants such as paroxetine (Lôo et al., 2002).

Other 5-HT receptors such as 5HT₃ receptors may also contribute to the changes in 5-HT-induced DA release (Dremencov et al., 2006). Thus, antidepressant drugs that will block 5-HT_{2C} and activate 5-HT₃ receptors will probably restore 5-HT-induced DA release in the nucleus accumbens, and normalize depressive-like behaviour faster than classical antidepressant drugs (Dremencov et al., 2004, 2005, 2006). This suggestion agrees with clinical studies that demonstrated that mirtazapine, which acts on 5-HT_{2C} and 5-HT₃ receptors, and nefazodone, which acts on 5-HT_{2C}, and whose metabolite acts on 5-HT₃ receptors, are characterized by a more rapid onset of behavioural effects of treatment (Artigas et al., 2002). Further, valproic acid, carbamazepine and zonisamide, three anticonvulsant mood stabilizers, have been reported to preferentially increase DA release in the mPFC of rats, sharing a common mechanism of action mediated by 5-HT_{1A} receptor activation (Ichikawa and Meltzer, 1999b; Ichikawa et al., 2005). Thus, both anticonvulsant mood stabilizers and atypical APDs would be expected to ameliorate or prevent depression, at least in part, via reversal of decreased prefrontal cortical activity by facilitating 5-HT_{1A} activation and its resultant increase in mPFC DA release. As already mentioned, a number of antidepressant agents, such as flibanserin (Invernizzi et al., 2003), ipsapirone (Wędzony et al., 1996), mirtazapine (Nakayama et al., 2004), fluoxetine and buspirone (Gobert et al., 1999; Sakaue et al., 2000), all drugs showing

agonistic properties at 5-HT_{1A} receptors, raised extracellular DA in mPFC, and were markedly attenuated by pretreatment with WAY 100635. Also, the combination of atypical APDs in addition to 5-HT reuptake inhibitors has recently proven to be beneficial in a number of neuropsychiatric disorders, such as resistant depression, schizophrenia and obsessive-compulsive disorder, as this method markedly potentiates mPFC DA release elicited by the administration of a single drug alone. Interestingly, acute combination of fluoxetine and olanzapine caused a synergistic and selective effect on the extracellular concentration of DA in the mPFC (Zhang et al., 2000; Koch et al., 2004), and this regional selectivity may account for the mood-stabilizing properties of these drugs. Nevertheless, the combination of long-term fluoxetine with acute olanzapine did not show the synergistic effect on the extracellular concentration of DA observed following acute administration (Amargós-Bosch et al., 2005), suggesting that the therapeutic benefit of this pharmacological combination may not be associated with changes in the cortical concentration of monoamines, but with postsynaptic blockade of monoaminergic receptors. Thus, activation of 5-HT_{1A} receptors secondary to the combined blockade of 5-HT_{2A/2C} and D_{2/3} receptors seems to be relevant for this action (Gobert et al., 1997, 1999; Gobert and Millan, 1999a; Zhang et al., 2000; Yoshino et al., 2002, 2004; Denys et al., 2004; Koch et al., 2004; Ago et al., 2005; Amargós-Bosch et al., 2005; Huang et al., 2006; Bortolozzi et al., 2007).

Schizophrenia

Both hypo- and hyperfunction of dopaminergic systems may occur in schizophrenic patients, perhaps even simultaneously, albeit in a region-specific manner (Davis et al., 1991; Svensson et al., 1993, 1995). Thus, whereas a dopaminergic hyperfunction of the mesolimbic system may underlie the development of positive symptoms, a dopaminergic hypofunction of the cortical projections may well be related to the negative symptomatology in schizophrenia. Given the critical role of cortical DA in cognitive functioning (Arnsten et al., 1994; Sawaguchi and Goldman-Rakic,

1994), the hypothesized cortical DA hypofunction may therefore also be implicated in the cognitive disturbances frequently experienced by schizophrenic patients. Hence, it appears likely that both the negative symptoms and the cognitive disturbances of schizophrenia may be associated with a hypofunction of the mesocortical DA system.

Currently used APDs are usually divided into two main classes, on the basis of their liability to induce neurological side-effects after long-term treatment. Drugs defined as typical APDs (e.g. chlorpromazine, haloperidol and trifluopromazine) are known to induce, following repeated administration, various EPS including Parkinson-like syndrome and tardive dyskinesia (Meltzer and Nash, 1991). On the other hand, chronic treatment with atypical APDs (e.g. clozapine, risperidone, sertindole and zotepine) is associated with a low incidence of neurological side-effects (Meltzer and Nash, 1991). Moreover, atypical APDs do not increase plasma prolactin levels in humans (Meltzer and Nash, 1991). The hypothesis that typical antipsychotics produce their clinical effects, as well as EPS, by blocking DA D₂ receptors in the mesolimbic and nigrostriatal systems, respectively (Meltzer and Nash, 1991), is now generally accepted. In contrast, the mechanisms responsible for the clinical effects of atypical APDs are still not clear. The most relevant hypothesis on the mode of action of the atypical antipsychotics is that their action depends on their interaction with central 5-HT_{2A} or 5-HT_{2C} receptor subtypes, more than with D₂ receptors (Meltzer et al., 1989; Meltzer and Nash, 1991; Roth et al., 1992). Numerous studies show that several APDs exhibit appreciable affinity for central 5-HT₂ receptors (Meltzer and Nash, 1991; Schotte et al., 1996) and induce significant blockade of these receptors in the human brain (Farde et al., 1995). Early clinical studies indicated that the selective 5-HT_{2A/2C} receptor antagonist ritanserin (Leysen et al., 1985; Schotte et al., 1989) could ameliorate negative symptoms as well as attenuate exciting EPS in schizophrenics treated with classical APDs (Bersani et al., 1990; Miller et al., 1990). The importance of 5-HT₂ receptor antagonism in the pharmacology of schizophrenia is further underlined by the fact that clozapine is indeed a

potent 5-HT_{2A} receptor antagonist and exhibits a high ratio of 5-HT_{2A} to D₂ receptor affinities (Schmidt et al., 1995; Ashby and Wang, 1996). In fact, by examining in vitro receptor binding data, Meltzer et al. (1989) found that typical and atypical antipsychotics could be distinguished on the basis of their 5-HT_{2A} to D₂ receptor binding ratios. Accordingly, they suggested that the mechanism of action of atypical APDs is based on their ability to achieve a balanced 5-HT_{2A} to D₂ receptor antagonistic action and not on their absolute affinity for these receptors per se. Such hypotheses have given momentum to the development of novel APDs with combined antiserotonergic and antidopaminergic properties. Indeed, agents acting at multi-receptor sites appear to be more promising as APDs, and recent data show that blockade of DA receptors and combined antagonism at 5-HT_{2A} as well as 5-HT_{2C} receptors may be involved in the therapeutic effects of novel antipsychotics (Meltzer, 1999; Bonaccorso et al., 2002; Jones and Blackburn, 2002). In this respect, it is noteworthy to mention recent data showing that atypical APDs (clozapine, sertindole, olanzapine, ziprasidone, risperidone, zotepine, tiospirone, fluperlapine and tenilapine), which produce little or no EPS while improving negative symptoms of schizophrenia, exert substantial inverse agonist activity at 5-HT_{2C} receptors (Herrick-Davis et al., 2000; Rauser et al., 2001). Thus, 5-HT_{2C} receptor inverse agonism might underlie the unique clinical properties of atypical APDs (Herrick-Davis et al., 2000).

Antagonism at 5-HT_{2C} receptors by several antipsychotics was also observed in vivo. Indeed, clozapine produced an increase in extracellular levels of DA in the nucleus accumbens (Di Matteo et al., 2002; Shilliam and Dawson, 2005), reversed the inhibition of accumbal DA release induced by the 5-HT_{2C} agonist RO 60-0175 (Di Matteo et al., 2002) and blocked the hypolocomotion induced by the 5-HT_{2C} agonist mCPP (Prinssen et al., 2000). It is worth noting that clozapine, like several atypical APDs, behaves as a 5-HT_{2C} inverse agonist in heterologous expression systems in vitro (Herrick-Davis et al., 2000; Rauser et al., 2001; Navailles et al., 2006a) and in vivo (Navailles et al., 2006a). Thus, the 5-HT_{2C} receptor inverse

agonism might underlie the unique clinical properties of atypical APDs (Herrick-Davis et al., 2000; Navailles et al., 2006a). The modification of 5-HT_{2C} receptors constitutive activity may also participate in the effects of the typical APD haloperidol. Indeed, it has been reported that the increase in striatal DA release induced by haloperidol is dramatically potentiated by the 5-HT_{2C} inverse agonist SB 206553 (Navailles et al., 2006a). Therefore, bearing in mind that haloperidol does not bind to 5-HT_{2C} receptors, it was suggested that it could act at the level of a common effector pathway (Navailles et al., 2006a).

A preferential increase of DA release in the mPFC seems to be a common mechanism of action of atypical APDs, an effect which might be relevant for their therapeutic action on negative symptoms of schizophrenia (Kuroki et al., 1999b). In this respect, it is important to note that the selective 5-HT_{2C} receptor antagonist SB 242084 (Kennett et al., 1997) markedly increases DA release in the frontal cortex of awake rats (Millan et al., 1998a; Gobert et al., 2000). Thus, it is possible to argue that blockade of 5-HT_{2C} receptors might contribute to the preferential effect of atypical antipsychotics on DA release in the prefrontal cortex. Interestingly, there is pre-clinical evidence indicating that 5-HT_{2C} receptor blockade is responsible for reducing EPS: 5-HT_{2C} but not 5-HT_{2A} receptor antagonists were capable of inhibiting haloperidol-induced catalepsy in rats (Reavill et al., 1999). On the other hand, the blockade of DA-ergic neurotransmission in the nucleus accumbens via D₂ receptor antagonism or partial agonism is considered the primary mechanism underlying antipsychotic efficacy for the positive symptoms (i.e. hallucinations, delusions and thought disorder) of schizophrenia. Thus, an alternative approach to blocking DA D₂ receptors may be to reduce the activity of the mesolimbic pathway without affecting that of the nigrostriatal system, thus avoiding potential EPS liabilities. The selective effects shown by the 5-HT_{2C} receptor agonists on the mesolimbic DA pathway suggest that 5-HT_{2C} receptor agonists should have antipsychotic efficacy without the EPS associated with typical antipsychotics. To this end, recently, the antipsychotic efficacy of the selective 5-HT_{2C}

receptor agonist WAY-163909 was preclinically evaluated by in vivo microdialysis, electrophysiology and various animal models of schizophrenia (Marquis et al., 2007), showing selectivity for the mesolimbic system and an interesting profile similar to that of an atypical antipsychotic, when given acutely or chronically in mice and rats, facilitating cortical DA-ergic neurotransmission and reducing that of the nucleus accumbens, without affecting the nigrostriatal DA activity.

Furthermore, a large number of novel atypical antipsychotic compounds, in various stages of development, show some degree of 5-HT_{1A} agonism (see Meltzer et al., 2003; De Oliveira and Juruena, 2006 for review). Thus, the 5-HT_{1A} receptor subtype plays an important role in controlling monoaminergic activity. This receptor subtype can be considered as functionally antagonistic to the 5-HT_{2A} receptor, at both the presynaptic and the postsynaptic levels, and this suggests that agonists at 5-HT_{1A} receptors may modulate DA-ergic neurotransmission in the brain in a similar fashion to 5-HT_{2A} receptor antagonists (Meltzer et al., 2003). As previously discussed, the superior clinical efficacy of clozapine, and that of numerous other atypical APDs, may be related to their ability to selectively increase DA release in the prefrontal cortex and hippocampus, rather than in the nucleus accumbens and striatum, in part due to 5-HT_{1A} receptors stimulation. Recent research has shown that 5-HT_{1A} agonism may be an important consequence of 5-HT_{2A} antagonism (Ichikawa et al., 2001b; Bonaccorso et al., 2002), and substitution of 5-HT_{1A} agonism for 5-HT_{2A} antagonism may also produce an atypical APD when coupled with weak D₂ antagonism (Meltzer et al., 2003; De Oliveira and Juruena, 2006). In this respect, combining antagonist/partial agonist activity at DA D₂ and agonist activity at 5-HT_{1A} receptors is one of the approaches that has recently been chosen to develop the new generation of antipsychotics, including bifeprunox, SSR181507 and SLV313, in that 5-HT_{1A} receptor activation greatly reduces or prevents the cataleptogenic potential of these novel antipsychotics (Claustre et al., 2003; Assié et al., 2005; McCreary et al., 2007). Therefore, a combination of these properties may normalize DA tone and

thus improve cognitive dysfunction of psychotic illness.

Parkinson's disease

The major pathology in PD is the degeneration of pigmented DA-producing neurons, particularly within the SNc, which causes a consequent reduction of DA levels in the striatum, and changes in the basal ganglia–thalamo-cortical network activity (Nicholson and Brotchie, 2002; Esposito et al., 2007a, b, c; Di Giovanni, 2008; Utter and Basso, 2008). The neural mechanisms underlying the generation of Parkinsonian symptoms are thought to involve reduced activation of primary motor and premotor cortex and supplementary motor areas, secondary to overactivation of the output regions of the basal ganglia, i.e. SNr and globus pallidus internus (GPi) (Albin et al., 1989), largely because of excessive excitatory drive from the subthalamic nucleus (STN), consequent to DA loss in the striatum (Nicholson and Brotchie, 2002; Utter and Basso, 2008). Therapy for PD consists mainly of amelioration of the symptoms with classical dopaminomimetics (Hagan et al., 1997). This treatment, however, is characterized by declining efficacy and the occurrence of disabling side-effects (Agid, 1998). Functional inhibition of GPi or STN has provided an alternative to lesioning, by deep brain stimulation associated with modest side-effects (Rodriguez et al., 1998). Since serotonergic projections from the DR nuclei innervate all components of the basal ganglia circuitry (Nicholson and Brotchie, 2002; Utter and Basso, 2008), it is likely that 5-HT plays a role in regulating the basal ganglia's activities, and of particular interest with respect to the development of new treatments for PD are 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors (Nicholson and Brotchie, 2002; Di Giovanni et al., 2006b). Stimulation of 5-HT_{1A} receptors might be expected to reduce 5-HT release in several brain regions, including the basal ganglia, and reduce the activity of glutamatergic inputs to the striatum, that may have both anti-Parkinsonian and anti-dyskinetic actions (Nicholson and Brotchie, 2002). Moreover, stimulation of striatal 5-HT_{1B} receptors may modulate levodopa metabolism to DA in

5-HT terminals (Knobelman et al., 2000). 5-HT_{1B} receptors in the pallidum and SN reduce GABA release, thus having either anti-Parkinsonian or antidyskinetic actions (Nicholson and Brotchie, 2002). Another interesting application of the data regarding the functional role of 5-HT_{2C} receptors in the basal ganglia is the possible use of 5-HT_{2C} receptor antagonists in the treatment of PD, and 5-HT_{2C} agonists to reduce the problems of levodopa-induced dyskinesia (Fox and Brotchie, 1999; Nicholson and Brotchie, 2002). As already mentioned, 5-HT_{2C} receptors are located in the SNr and medial segment of the pallidal complex in the rat and human brain (Azmitia and Segal, 1978; Pasqualetti et al., 1999), and enhanced 5-HT_{2C} receptor-mediated transmission within the output regions of the basal ganglia in Parkinsonism appears to contribute to their overactivity (Fox and Brotchie, 1999). In addition, 5-HT_{2C}-like receptor binding is increased in a rat model of Parkinsonism (Radja et al., 1993) and in human Parkinsonian patients (Fox and Brotchie, 2000a). Interestingly, systemic administration of SB 206553 enhanced the anti-Parkinsonian action of the DA D₁ and D₂ agonists in the 6-hydroxydopamine (6-OHDA)-lesioned rats (Fox et al., 1998; Fox and Brotchie, 2000b), suggesting that the use of a 5-HT_{2C} receptor antagonist in combination with a DA receptor agonist may reduce the reliance on DA replacement therapies and may thus reduce the problems associated with long-term use of currently available anti-Parkinsonian agents (Fox and Brotchie, 1999).

Drugs of abuse

Substantial evidence indicates that the mesolimbic pathway, particularly the dopaminergic system innervating accumbal areas, is implicated in the reward value of both natural and drug reinforcers, such as sexual behaviour or psychostimulants, respectively (Di Chiara and Imperato, 1988; Koob, 1992; Spanagel and Weiss, 1999). Therefore, blocking or stimulating several 5-HT receptor subtypes, including the 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT₃ subtypes, modulates both the neurochemical and the behavioural effects of addictive

drugs. 5-HT₃ receptors have been shown to play a permissive role in the effects of both cocaine and ethanol. In contrast, 5-HT₃ antagonism counteracted the increase of accumbal DA release induced by various addictive drugs such as ethanol, nicotine, morphine or cocaine (Carboni et al., 1989; McNeish et al., 1993; Pei et al., 1993; Kankaanpää et al., 1996, 2002; De Deurwaerdère et al., 2005). Further, 5-HT_{1B} receptor agonists potentiated both cocaine- and ethanol-induced increase of mesolimbic DA release (Parsons et al., 1999; O'Dell and Parsons, 2004; Yan et al., 2005), and blockade of VTA 5-HT_{1B} receptors attenuated ethanol-induced increases in extracellular DA concentrations in both the VTA and the ipsilateral nucleus accumbens (Yan et al., 2005). These studies suggest that 5-HT_{1B} and/or 5-HT₃ receptor antagonism could be beneficial in treating psychostimulant abuse. 5-HT_{2A} and 5-HT_{1A} receptor subtypes have also been implicated in modulating responses to psychostimulants and may play a role in their rewarding effects. The fact that addictive drugs act through different cellular mechanisms leads to the possibility that their effects on DA release could be modulated differentially by each of the 5-HT_{2A} or 5-HT_{2C} receptor subtypes. For example, it has been reported that the increased locomotor activity, as well as the accumbal DA release, elicited by phencyclidine is further enhanced by the blockade of 5-HT_{2C} receptors (Hutson et al., 2000), while antagonism at 5-HT_{2A} receptors had opposite effects (Maurel-Remy et al., 1995). A similar picture emerges when considering the influence of these receptors on MDMA (ecstasy)-induced effects on DA neuron activity. Thus, the selective 5-HT_{2A} antagonist MDL 100,907 significantly reduced hyperlocomotion and stimulated the DA release produced by MDMA while the selective 5-HT_{2C} antagonists SB 242084 and SB 206553 potentiated it (Schmidt et al., 1992; Kehne et al., 1996; Bankson and Cunningham, 2002; Fletcher et al., 2002a, b).

It was recently found that SB 206553 administration potentiates both the enhancement of DA release in the nucleus accumbens and striatum, and the increased DA neuron firing rate induced by

morphine in both the VTA and the SNc (Porras et al., 2002b). Consistent with these findings, stimulation of central 5-HT_{2C} receptors has been shown to inhibit morphine-induced increase in DA release in the nucleus accumbens of freely moving rats (Willins and Meltzer, 1998). A series of studies showed that blockade of 5-HT_{2A} or 5-HT_{2C} receptors had opposite effects on cocaine-induced locomotor activity. Thus, 5-HT_{2A} receptor blockade with M100,907 attenuated cocaine-induced locomotion, whereas 5-HT_{2C} blockade with SB 242084 or SB 206553 enhanced cocaine-induced activity (McCreary and Cunningham, 1999; O'Neill et al., 1999; McMahon and Cunningham, 2001; Fletcher et al., 2002a, b). Consistent with these data obtained in rats, 5-HT_{2C} receptor null mutant mice showed enhanced cocaine-induced elevations of DA levels in the nucleus accumbens, and marked increase in locomotor response to cocaine as compared to wild-type mice, suggesting that selective 5-HT_{2C} receptor agonist treatments may represent a promising novel approach for treating cocaine abuse and dependence (Rocha et al., 2002). In line with this hypothesis, it was previously found that RO 60-0175 reduced cocaine-reinforced behaviour by stimulating 5-HT_{2C} receptors (Grottick et al., 2000). Moreover, these authors also showed that RO 60-0175 reduced ethanol- and nicotine-induced self-administration and hyperactivity (Grottick et al., 2001; Tomkins et al., 2002). Consistent with this evidence, we showed that the selective activation of 5-HT_{2C} receptors by RO 60-0175 blocks the stimulatory action of nicotine on SNc DA neuronal activity and DA release in the corpus striatum (Di Matteo et al., 2004; Pierucci et al., 2004). The mesolimbic DA system appeared to be less sensitive to the inhibitory effect of 5-HT_{2C} receptors activation on nicotine-induced stimulation; indeed, a higher dose of RO 60-0175 was necessary to prevent the enhancement of VTA DA neuronal firing elicited by acute nicotine. Furthermore, pretreatment with the 5-HT_{2C} agonist did not affect nicotine-induced DA release in the nucleus accumbens (Di Matteo et al., 2004). Interestingly, in animals treated repeatedly with nicotine, pretreatment with RO 60-0175 reproduced the same

pattern of effects on the enhancement in DA neuronal firing caused by challenge with nicotine, and as a result was effective only at a higher dose in preventing nicotine excitation in the VTA compared to the SNc. Furthermore, the 5-HT_{2C} receptors agonist counteracted nicotine-induced DA release both in the striatum and in the nucleus accumbens in rats chronically treated with this alkaloid, even if this effect was observed only with the highest dose of RO 60-0175 (Di Matteo et al., 2004; Pierucci et al., 2004). Therefore, we hypothesized that after repeated nicotine exposure, an up-regulation of 5-HT_{2C} receptors occurs only in the DA mesolimbic system and the blocking of its hyperfunction by 5-HT_{2C} receptor activation might be a useful approach in reducing nicotine reward, and eventually helping in smoking cessation.

Conclusion

Serotonergic and dopaminergic systems are closely related in the CNS, and the involvement of 5-HT receptors in the control of central DA activity is now well established. Recent evidence suggests that dysfunction of dopaminergic and serotonergic neurotransmitter systems contributes to various disorders including depression, schizophrenia, PD and drug abuse. Thus, the use of a complementary dialysis and electrophysiological approach, together with several highly selective ligands, has permitted important insights into the complex pattern of reciprocal interactions via which multiples classes of auto- and heteroreceptors control the activity of central dopaminergic pathways. With the exception of the constitutively active 5-HT_{2C} receptor, the others 5-HT receptor subtypes do not appear to tonically modulate DA-ergic activity, as evidenced by the lack of effect of antagonist treatments alone. On the other hand, 5-HT receptors are, nearly all, capable of regulating DA activity when 5-HT tone is elevated, or when they are stimulated by exogenous agonists. These effects are often indirect and mediated by complex neuronal circuitry involving other transmitters. For example, as previously

described, 5-HT_{2A} and 5-HT_{1A} receptors are thought to be localized to pyramidal glutamatergic neurons in the mPFC, and to regulate DA function through 'long-loop' feedback to the VTA. Likewise, there is evidence that 5-HT_{2C} and 5-HT_{1B} receptors in the VTA regulate mesocorticolimbic DA neurons indirectly by influencing GABA release from their host cells. These data facilitate interpretation of the influence on central DA-ergic systems exerted by 5-HT and diverse classes of antidepressant, antipsychotic and psychostimulant agents, and suggest numerous possible receptorial strategies for modulation (potentiation) of their therapeutic actions. The majority of the effects described herein were acquired on acute drug administration. This approach is eminently suitable to the characterization of the functional roles of various auto- and heteroreceptor subtypes. However, inasmuch as the majority of these therapeutic agents are often administered chronically, and may trigger adaptive changes, it would be interesting to expand the present observations with studies of long-term drug administration.

Abbreviations

5-HT	serotonin
6-OHDA	6-hydroxydopamine
APDs	antipsychotic drugs
CCK	cholecystokinin
CNS	central nervous system
DA	dopamine
DR, MR	dorsal raphe, median raphe
EPS	extrapyramidal side-effects
GABA	γ -amino- <i>n</i> -butyric acid
GPI	globus pallidus internus
HPLC	high-performance liquid chromatography
mPFC	medial prefrontal cortex
SN, SNc, SNr	substantia nigra, substantia nigra pars compacta, substantia nigra reticulata
SSRIs	selective serotonin reuptake inhibitors
STN	subthalamic nucleus
VTA	ventral tegmental area

Acknowledgement

The authors wish to thank Ms. Barbara Mariani for her help in preparing the manuscript.

References

- Abramowski, D., Rigo, M., Due, D., Hoyer, D. and Staufenbiel, M. (1995) Localization of 5-hydroxytryptamine_{2C} receptor protein in human and rat brain using specific antisera. *Neuropharmacology*, 35: 1635–1645.
- Agid, Y. (1998) Levodopa: is toxicity a myth? *Neurology*, 50: 858–863.
- Ago, Y., Koyama, Y., Baba, A. and Matsuda, T. (2003) Regulation by 5-HT_{1A} receptors of the in vivo release of 5-HT and DA in mouse frontal cortex. *Neuropharmacology*, 45: 1050–1056.
- Ago, Y., Nakamura, S., Baba, A. and Matsuda, T. (2005) Sulpiride in combination with fluvoxamine increases in vivo dopamine release selectively in rat prefrontal cortex. *Neuropsychopharmacology*, 1: 43–51.
- Albin, R., Young, A.B. and Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.*, 12: 366–375.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113: 296–320.
- Alex, K.D., Yavanian, G.J., McFarlane, H.G., Pluto, C.P. and Pehek, E.A. (2005) Modulation of dopamine release by striatal 5-HT_{2C} receptors. *Synapse*, 55: 242–251.
- Amargós-Bosch, M., Artigas, F. and Adell, A. (2005) Effects of acute olanzapine after sustained fluoxetine on extracellular monoamine levels in the rat medial prefrontal cortex. *Eur. J. Pharmacol.*, 516: 235–238.
- Amargós-Bosch, M., Bortolozzi, A., Puig, M.V., Serrats, J., Adell, A., Celada, P., Toth, M., Mengod, G. and Artigas, F. (2004) Co-expression and in vivo interaction of serotonin_{1A} and serotonin_{2A} receptors in prefrontal cortex. *Cereb. Cortex*, 14: 281–299.
- Andersson, J.L., Nomikos, G.G., Marcus, M., Hertel, P., Mathé, J.M. and Svensson, T.H. (1995) Ritanserin potentiates the stimulatory effects of raclopride on neuronal activity and dopamine release selectively in the mesolimbic dopaminergic system. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 352: 374–385.
- Arborelius, L., Nokimos, G.G., Hacksell, U. and Svensson, T.H. (1993) (*R*)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. *Acta Physiol. Scand.*, 148: 465–466.
- Arnsten, A.F., Cai, J.X., Murphy, B.L. and Goldman-Rakic, P.S. (1994) Dopamine D₁ receptor mechanisms in the cognitive performance of young adult and aged monkeys. *Psychopharmacology*, 116: 143–151.

- Artigas, F., Nutt, D.J. and Shelton, R. (2002) Mechanism of action of antidepressants. *Psychopharmacol. Bull.*, 36(Suppl. 2): 123–132.
- Ashby, C.R. and Wang, R.Y. (1996) Pharmacological actions of the atypical antipsychotic drug clozapine. A review. *Synapse*, 24: 349–394.
- Assié, M.-B., Ravailhe, V., Faucillon, V. and Newman-Tancredi, A. (2005) Contrasting contribution of 5-hydroxytryptamine 1A receptor activation to neurochemical profile of novel antipsychotics: frontocortical dopamine and hippocampal serotonin release in rat brain. *J. Pharmacol. Exp. Ther.*, 315: 265–272.
- Auclair, A., Blanc, G., Glowinski, J. and Tassin, J.-P. (2004) Role of serotonin_{2A} receptors in the D-amphetamine-induced release of dopamine: comparison with previous data on α 1b-adrenergic receptors. *J. Neurochem.*, 91: 318–326.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179: 641–668.
- Bankson, G.M. and Cunningham, K.A. (2002) Pharmacological studies of the acute effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT_{1B/1D} and 5-HT₂ receptors. *Neuropsychopharmacology*, 26: 40–52.
- Bankson, M.G. and Yamamoto, B.K. (2004) Serotonin–GABA interactions modulate MDMA-induced mesolimbic dopamine release. *J. Neurochem.*, 91: 852–859.
- Bannon, M.J. and Roth, R.H. (1983) Pharmacology of mesocortical dopamine neurons. *Pharmacol. Rev.*, 35: 53–68.
- Barker, E.L. and Sanders-Bush, E. (1993) 5-Hydroxytryptamine_{1C} receptor density and mRNA levels in choroid plexus epithelial cells after treatment with mianserin and (–)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane. *Mol. Pharmacol.*, 44: 725–730.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38: 1083–1152.
- Baxter, G.S., Kennett, G.A., Blaney, F. and Blackburn, T. (1995) 5-HT₂ receptor subtypes: a family reunited? *Trends. Pharmacol. Sci.*, 16: 105–110.
- Benloucif, S., Keegan, M.J. and Galloway, M.P. (1993) Serotonin-facilitated dopamine release in vivo: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 265: 373–377.
- Bentué-Ferrer, D., Reymann, J.-M., Rousselle, J.-C., Massot, O., Bourin, M., Allain, H. and Fillion, G. (1998) 5-HT-moduline, a 5-HT_{1B/1D} receptor endogenous modulator, interacts with dopamine release measured in vivo by microdialysis. *Eur. J. Pharmacol.*, 358: 129–137.
- Berg, K.A., Navailles, S., Sanchez, T.A., Silva, Y.M., Wood, M.D., Spampinato, U. and Clarke, W.P. (2006) Differential effects of 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]-carbamoyl]-6-trifluoromethylindone (SB 243213) on 5-hydroxytryptamine_{2C} receptor-mediated responses. *J. Pharmacol. Exp. Ther.*, 319: 260–268.
- Bersani, G., Grispi, A., Marini, S., Pasini, A., Valducci, M. and Ciani, N. (1990) 5-HT₂ antagonist ritanserin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. *Clin. Neuropharmacol.*, 13: 500–506.
- Blackburn, T.P., Minabe, Y., Middlemiss, D.N., Shirayama, Y., Hashimoto, K. and Ashby, C.R. (2002) Effect of acute and chronic administration of the selective 5-HT_{2C} receptor antagonist SB-243213 on midbrain dopamine neurons in the rat: an in vivo extracellular single cell study. *Synapse*, 46: 129–139.
- Boess, F.G. and Martin, I.L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology*, 33: 275–317.
- Bonaccorso, S., Meltzer, H.Y., Li, Z., Dai, J., Alboszta, A.R. and Ichikawa, J. (2002) SR46349-B, a 5-HT_{2A/2C} receptor antagonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Neuropsychopharmacology*, 27: 430–441.
- Bonasera, S.J. and Tecott, L.H. (2000) Mouse models of serotonin receptor function: toward a genetic dissection of serotonin systems. *Pharmacol. Ther.*, 88: 133–142.
- Bonhomme, N., De Deurwaerdere, P., Le Moal, M. and Spampinato, U. (1995) Evidence for 5-HT₄ receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin, a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology*, 34: 269–279.
- Bortolozzi, A., Díaz-Mataix, L., Scorza, M.C., Celada, P. and Artigas, F. (2005) The activation of 5-HT_{2A} receptors in prefrontal cortex enhances dopaminergic activity. *J. Neurochem.*, 95: 1597–1607.
- Bortolozzi, A., Díaz-Mataix, L., Toth, M., Celada, P. and Artigas, F. (2007) In vivo actions of aripiprazole on serotonergic and dopaminergic systems in rodent brain. *Psychopharmacology*, 191: 745–758.
- Boschert, U., Amara, A., Segu, L. and Hen, R. (1994) The mouse 5-hydroxytryptamine_{1B} receptor is localized predominantly on axon terminals. *Neuroscience*, 58: 167–182.
- Boulenguez, P., Rawlins, J.N.P., Chauveau, J., Joseph, M.H., Mitchell, S.N. and Gray, J.A. (1996) Modulation of dopamine release in the nucleus accumbens by 5-HT_{1B} agonists: involvement of the hippocampo-accumbens pathway. *Neuropharmacology*, 35: 1521–1529.
- Bowers, B.J., Henry, M.B., Thielen, R.J. and McBride, W.J. (2000) Serotonin 5-HT₂ receptor stimulation of dopamine release in the posterior but not anterior nucleus accumbens of the rat. *J. Neurochem.*, 75: 1625–1633.
- Brown, A.S. and Gershon, S. (1993) Dopamine and depression. *J. Neural Transm.*, 91: 75–109.
- Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D. and Palacios, J.M. (1994) Localization of 5-HT_{1B}, 5-HT_{1D} α , 5-HT_{1E} and 5-HT_{1F} receptor messenger RNA in rodent and primate brain. *Neuropharmacology*, 33: 367–386.
- Bruinvels, A.T., Palacios, J.M. and Hoyer, D. (1993) Autoradiographic characterisation and localisation of 5-HT_{1D} compared to 5-HT_{1B} binding sites in rat brain. *Naunyn-Schmiedeberg Arch. Pharmacol.*, 347: 569–582.

- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146: 286–297.
- Cabib, S. and Puglisi-Allegra, S. (1996) Stress, depression and the mesolimbic dopamine system. *Psychopharmacology*, 128: 331–342.
- Cadoni, C., Pinna, A., Russi, G., Consolo, S. and Di Chiara, G. (1995) Role of vesicular dopamine in the *in vivo* stimulation of striatal dopamine transmission by amphetamine: evidence from microdialysis and Fos immunohistochemistry. *Neuroscience*, 65: 1027–1039.
- Campbell, A., Kohl, R. and McBride, W. (1996) Serotonin-3 receptor and ethanol-stimulated somatodendritic dopamine release. *Alcohol*, 13: 569–574.
- Campbell, W. and McBride, W.J. (1995) Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens. *Pharmacol. Biochem. Behav.*, 51: 835–842.
- Carboni, E., Acquas, E., Frau, R. and Di Chiara, G. (1989) Differential inhibitory effects of a 5-HT₃ antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.*, 164: 515–519.
- Cervo, L., Grignaschi, G. and Samanin, R. (1990) The role of the mesolimbic dopaminergic system in the desipramine effect in the forced swimming test. *Eur. J. Pharmacol.*, 178: 129–133.
- Cervo, L., Pozzi, L. and Samanin, R. (1996) 5-HT₃ receptor antagonists do not modify cocaine place conditioning or the rise in extracellular dopamine in the nucleus accumbens of rats. *Pharmacol. Biochem. Behav.*, 55: 33–37.
- Cervo, L. and Samanin, R. (1987) Evidence that dopamine mechanisms in the nucleus accumbens are selectively involved in the effect of desipramine in the forced swimming test. *Neuropharmacology*, 26: 1469–1472.
- Cervo, L. and Samanin, R. (1988) Repeated treatment with imipramine and amitriptyline reduced the immobility of rats in the swimming test by enhancing dopamine mechanisms in the nucleus accumbens. *J. Pharm. Pharmacol.*, 40: 155–156.
- Chen, J., Paredes, W., Van Praag, H.M., Lowinson, J.H. and Gardner, E.L. (1992) Presynaptic dopamine release is enhanced by 5-HT₃ receptor activation in medial prefrontal cortex of freely moving rats. *Synapse*, 10: 264–266.
- Chen, J., van Praag, H.M. and Gardner, E.L. (1991) Activation of 5-HT₃ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. *Brain Res.*, 543: 354–357.
- Chung, Y.-C., Li, Z., Dai, J., Meltzer, H.Y. and Ichikawa, J. (2004) Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 1023: 54–63.
- Claustre, Y., De Peretti, D., Brun, P., Gueudet, C., Allouard, N., Alonso, R., Lourdelet, J., Oblin, A., Damoiseau, G., Françon, D., Suaud-Chagny, M.-F., Steinberg, R., Sevrin, M., Schoemaker, H., George, P., Soubrié, P. and Scatton, B. (2003) SSR181507, a dopamine D₂ receptor antagonist and 5-HT_{1A} receptor agonist. I: neurochemical and electrophysiological profile. *Neuropsychopharmacology*, 28: 2064–2076.
- Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P. and Fone, K.C.F. (2000) Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. *Neuropharmacology*, 39: 123–132.
- D'Aquila, P.S., Collu, M., Gessa, G.L. and Serra, G. (2000) The role of dopamine in the mechanism of action of antidepressant drugs. *Eur. J. Pharmacol.*, 405: 365–373.
- Dahlström, A. and Fuxe, K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.*, 62: 1–55.
- Davis, K.L., Kahn, R.S., Ko, G. and Davidson, M. (1991) Dopamine in schizophrenia: a review and reconceptualization. *Am. J. Psychiatry*, 148: 1474–1486.
- Dawson, L.A. and Li, P. (2003) Effects of 5-HT₆ receptor blockade on the neurochemical outcome of antidepressant treatment in the frontal cortex of the rat. *J. Neural Transm.*, 110: 577–590.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2000) *In vivo* effects of the 5-HT₆ antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate. *Br. J. Pharmacol.*, 130: 23–26.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2001) The 5-HT₆ receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. *Neuropsychopharmacology*, 25: 662–668.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2003) Potentiation of amphetamine-induced changes in dopamine and 5-HT by a 5-HT₆ receptor antagonist. *Brain Res. Bull.*, 59: 513–521.
- De Deurwaerdère, P., L'hirondel, M., Bonhomme, N., Lucas, G., Cheramy, A. and Spampinato, U. (1997) Serotonin stimulation of 5-HT₄ receptors indirectly enhances *in vivo* dopamine release in the rat striatum. *J. Neurochem.*, 68: 195–203.
- De Deurwaerdère, P., Moison, D., Navailles, S., Porras, G. and Spampinato, U. (2005) Regionally and functionally distinct serotonin₃ receptors control *in vivo* dopamine outflow in the rat nucleus accumbens. *J. Neurochem.*, 94: 140–149.
- De Deurwaerdère, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits *in vivo* dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24: 3235–3241.
- De Deurwaerdère, P. and Spampinato, U. (1999) Role of serotonin_{2A} and serotonin_{2B/2C} receptor subtypes in the control of accumbal and striatal dopamine release elicited *in vivo* by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73: 1033–1042.
- De Deurwaerdère, P., Stinus, L. and Spampinato, U. (1998) Opposite change of *in vivo* dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT₃ receptors. *J. Neurosci.*, 18: 6528–6538.
- De Oliveira, I.R. and Juruena, M.F. (2006) Treatment of psychosis: 30 years of progress. *J. Clin. Pharm. Ther.*, 31: 523–534.
- Denys, D., Klomp makers, A.A. and Westenberg, H.G. (2004) Synergistic dopamine increase in the rat prefrontal cortex with the combination of quetiapine and fluvoxamine. *Psychopharmacology*, 176: 195–203.

- Devaud, L.L., Hollingsworth, E.B. and Cooper, B.R. (1992) Alterations in extracellular and tissue levels of biogenic amines in rat brain induced by the serotonin₂ receptor antagonist, ritanserin. *J. Neurochem.*, 59: 1459–1466.
- Di Chiara, G. and Imperato, A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl. Acad. Sci. U.S.A.*, 85: 5274–5278.
- Di Chiara, G. and North, R.A. (1992) Neurobiology of opiate abuse. *Trends Pharmacol. Sci.*, 13: 185–192.
- Di Giovanni, G. (2008) Will it ever become possible to prevent dopaminergic neuronal degeneration? *CNS Neurol. Disord. Drug Targets*, 7(1): 28–44.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin_{2C/2B} receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91: 587–597.
- Di Giovanni, G., Di Matteo, V., Di Mascio, M. and Esposito, E. (2000) Preferential modulation of mesolimbic versus nigrostriatal dopaminergic function by serotonin_{2C/2B} receptor agonists: a combined in vivo electrophysiological and microdialysis study. *Synapse*, 35: 53–61.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) *m*-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience*, 103: 111–116.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006a) Central serotonin_{2C} receptor: from physiology to pathology. *Curr. Top. Med. Chem.*, 6: 1909–1925.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006b) Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT_{2C} receptor be a new target for therapeutic strategies? *Curr. Med. Chem.*, 13: 3069–3081.
- Di Mascio, M., Di Giovanni, G., Di Matteo, V., Prisco, S. and Esposito, E. (1998) Selective serotonin reuptake inhibitors reduce the spontaneous activity of dopaminergic neurons in the ventral tegmental area. *Brain Res. Bull.*, 46: 547–554.
- Di Matteo, V., Cacchio, M., Di Giulio, C., Di Giovanni, G. and Esposito, E. (2002) Biochemical evidence that the atypical antipsychotic drugs clozapine and risperidone block 5-HT_{2C} receptors in vivo. *Pharmacol. Biochem. Behav.*, 71: 607–613.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22: 229–232.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1998) Selective blockade of serotonin_{2C/2B} receptors enhances dopamine release in the rat nucleus accumbens. *Neuropharmacology*, 37: 265–272.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38: 1195–1205.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000a) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin_{2C} receptors. *Brain Res.*, 865: 85–90.
- Di Matteo, V., Di Giovanni, G. and Esposito, E. (2000b) SB 242084: a selective 5-HT_{2C} receptor antagonist. *CNS Drug Rev.*, 6: 195–205.
- Di Matteo, V., Di Mascio, M., Di Giovanni, G. and Esposito, E. (2000c) Acute administration of amitriptyline and mianserin increases dopamine release in the rat nucleus accumbens: possible involvement of serotonin_{2C} receptors. *Psychopharmacology*, 150: 45–51.
- Di Matteo, V., Pierucci, M. and Esposito, E. (2004) Selective stimulation of serotonin_{2C} receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. *J. Neurochem.*, 89: 418–429.
- Díaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25: 10831–10843.
- Doherty, M.D. and Pickel, V. (2000) Ultrastructural localization of serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.*, 864: 176–185.
- Doherty, M.D. and Pickel, V.M. (2001) Targeting of serotonin 1A receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain. *J. Comp. Neurol.*, 433: 390–400.
- Dremencov, E., Gispan-Herman, I., Rosenstein, M., Mendelman, A., Overstreet, D.H., Zohar, J. and Yadid, G. (2004) The serotonin–dopamine interaction is critical for fast-onset action of antidepressant treatment: in vivo studies in an animal model of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28: 141–147.
- Dremencov, E., Newman, M.E., Kinor, N., Blatman-Jan, G., Schindler, C.J., Overstreet, D.H. and Yadid, G. (2005) Hyperfunctionality of serotonin-2C receptor-mediated inhibition of accumbal dopamine release in an animal model of depression is reversed by antidepressant treatment. *Neuropharmacology*, 48: 34–42.
- Dremencov, E., Weizmann, Y., Kinor, N., Gispan-Herman, I. and Yadid, G. (2006) Modulation of dopamine transmission by 5HT_{2C} and 5HT₃ receptors: a role in the antidepressant response. *Curr. Drug Targets*, 7: 165–175.
- Duxon, M.S., Flanigan, T.P., Reavley, A.C., Baxter, G.S., Blackburn, T.P. and Fone, K.C.F. (1997) Evidence for expression of the 5-hydroxytryptamine_{2B} receptor protein in the rat central nervous system. *Neuroscience*, 76: 323–329.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.-F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384: 233–247.
- Eglen, R.M., Wong, E.H., Dumuis, A. and Bockaert, J. (1995) Central 5-HT₄ receptors. *Trends Pharmacol. Sci.*, 16: 391–398.
- Esposito, E., Di Matteo, V., Benigno, A., Pierucci, M., Crescimanno, G. and Di Giovanni, G. (2007a) Non-steroidal

- anti-inflammatory drugs in Parkinson's disease. *Exp. Neurol.*, 205(2): 295–312.
- Esposito, E., Di Matteo, V. and Di Giovanni, G. (2007b) Death in the substantia nigra: a motor tragedy. *Expert Rev. Neurother.*, 7(6): 677–697.
- Esposito, E., Di Matteo, V., Pierucci, M., Benigno, A. and Di Giovanni, G. (2007c) Role of central 5-HT_{2C} receptor in the control of basal ganglia functions. In: Di Giovanni G. (Ed.), *The Basal Ganglia Pathophysiology: Recent Advances*. Transworld Research Network, Trivandrum, pp. 97–127.
- Farde, L., Nyberg, S., Oxenstierna, G., Nakashima, Y., Halldin, C. and Ericsson, B. (1995) Positron emission tomography studies on D2 and 5-HT₂ receptor binding in risperidone-treated schizophrenic patients. *J. Clin. Psychopharmacol.*, 15: 19S–23S.
- Fibiger, H.C. (1995) Neurobiology of depression: focus on dopamine. In: Gessa G., Fratta W., Pani L. and Serra G. (Eds.), *Depression and Mania: From Neurobiology to Treatment*. Raven Press, New York, pp. 1–17.
- Flanigan, T.P., Reaveley, A.C., Carey, J.E. and Leslie, R.A. (1995) Evidence for the expression of the 5-HT_{2B} receptor mRNA in the rat brain. *Br. J. Pharmacol.*, 115: p. 369P.
- Fletcher, P.J., Korth, K.M., Robinson, S.R. and Baker, G.B. (2002a) Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement. *Psychopharmacology*, 162: 282–291.
- Fletcher, P.J., Phil, D., Grottick, A.J. and Higgins, G.A. (2002b) Differential effects of the 5-HT_{2A} receptor antagonist M100,907 and the 5-HT_{2C} receptor antagonist SB242,084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27: 576–586.
- Fox, S.H. and Brotchie, J.M. (1999) A role for 5-HT_{2C} receptor antagonists in the treatment of Parkinson's disease? *Drugs News Perspect.*, 12: 477–483.
- Fox, S.H. and Brotchie, J.M. (2000a) 5-HT_{2C} receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Mov. Disord.*, 15: 1064–1069.
- Fox, S.H. and Brotchie, J.M. (2000b) 5-HT_{2C} receptor antagonists enhance the behavioural response to dopamine D1 receptor agonists in the 6-hydroxydopamine-lesioned rat. *Eur. J. Pharmacol.*, 398: 59–64.
- Fox, S.H., Moser, B. and Brotchie, J.M. (1998) Behavioural effects of 5-HT_{2C} receptor antagonism in the substantia nigra zona reticulata of the 6-OHDA-lesioned rat model of Parkinson's disease. *Exp. Neurol.*, 151: 35–49.
- Frantz, K.J., Hansson, K.J., Stouffer, D.G. and Parsons, L.H. (2002) 5-HT₆ receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. *Neuropharmacology*, 42: 170–180.
- Galloway, M.P., Suchowski, C.S., Keegan, M.J. and Hjorth, S. (1993) Local infusion of the selective 5HT-1b agonist, CP-93,128 facilitates striatal dopamine release in vivo. *Synapse*, 15: 90–92.
- Gerard, C., El Mestikawy, S., Lebrand, C., Adrien, J., Ruat, M., Traffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23: 164–173.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and El Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 746: 207–219.
- Giorgetti, M. and Tecott, L. (2004) Contribution of 5-HT_{2C} receptors to multiple action of central serotonin systems. *Eur. J. Pharmacol.*, 488: 1–9.
- Gobert, A. and Millan, M.J. (1999a) Modulation of dialysate levels of dopamine, noradrenaline, and serotonin (5 HT) in the frontal cortex of freely-moving rats by (–)-pindolol alone and in association with 5-HT reuptake inhibitors: comparative roles of beta-adrenergic, 5-HT_{1A}, and 5-HT_{1B} receptors. *Neuropsychopharmacology*, 21: 268–284.
- Gobert, A. and Millan, M.J. (1999b) Serotonin (5-HT)_{2A} receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology*, 38: 315–317.
- Gobert, A., Rivet, J.M., Audinot, C., Newman-Tancredi, A.N., Cistarelli, L. and Millan, M.J. (1998) Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neuroscience*, 84: 413–429.
- Gobert, A., Rivet, J.M., Cistarelli, L., Melon, C. and Millan, M.J. (1999) Buspirone modulates basal and fluoxetine-stimulated dialysate levels of dopamine, noradrenaline and serotonin in the frontal cortex of freely moving rats: activation of serotonin_{1A} receptors and blockade of alpha₂-adrenergic receptors underlie its actions. *Neuroscience*, 93(4): 1251–1262.
- Gobert, A., Rivet, J.M., Cistarelli, L. and Millan, M.J. (1997) Buspirone enhances duloxetine- and fluoxetine-induced increases in dialysate levels of dopamine and noradrenaline, but not serotonin, in the frontal cortex of freely moving rats. *J. Neurochem.*, 68: 1326–1329.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.-P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36: 205–221.
- Grace, A. and Bunney, B. (1985) Dopamine. In: Rogawski M.A. and Barker J.L. (Eds.), *Neurotransmitter Action in the Vertebrate Nervous System*. Plenum Press, New York, pp. 285–319.
- Grottick, A.J., Corrigan, W.A. and Higgins, G.A. (2001) Activation of 5-HT_{2C} receptors reduces the locomotor and rewarding effects of nicotine. *Psychopharmacology*, 157: 292–298.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295: 1183–1191.

- Gudelsky, G.A., Yamamoto, B.K. and Nash, J.F. (1994) Potentiation of 3,4 methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT₂ receptor agonists. *Eur. J. Pharmacol.*, 264: 325–330.
- Hagan, J.J., Middlemiss, D.N., Sharp, P.C. and Poste, G.H. (1997) Parkinson's disease: prospects for improved drug therapy. *Trends Pharmacol. Sci.*, 18: 156–163.
- Hagino, Y. and Watanabe, M. (2002) Effects of clozapine on the efflux of serotonin and dopamine in the rat brain: the role of 5-HT_{1A} receptors. *Can. J. Physiol. Pharmacol.*, 80: 1158–1166.
- Hållbus, M., Magnusson, T. and Magnusson, O. (1997) Influence of 5-HT_{1B/1D} receptors on dopamine release in the guinea pig nucleus accumbens: a microdialysis study. *Neurosci. Lett.*, 225: 57–60.
- Herrick-Davis, K., Grinde, E. and Teitler, M. (2000) Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295: 226–232.
- Hertel, P., Nomikos, G.G., Iurlo, M. and Swensson, T.H. (1996) Risperidone: regional effects in vivo on release and metabolism of dopamine and serotonin in the rat brain. *Psychopharmacology*, 124: 74–86.
- Hervé, D., Pickel, V.M., Joh, T.H. and Beaudet, A. (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. *Brain Res.*, 435: 71–83.
- Higgins, G.A. and Fletcher, P.J. (2003) Serotonin and drug reward: focus on 5-HT_{2C} receptors. *Eur. J. Pharmacol.*, 480: 151–162.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Harting, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P.A. (1994) VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, 46: 157–203.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71: 533–554.
- Huang, M., Ichikawa, J., Li, Z., Dai, J. and Meltzer, H.Y. (2006) Augmentation by citalopram of risperidone-induced monoamine release in rat prefrontal cortex. *Psychopharmacology*, 185: 274–281.
- Hutson, P.H., Barton, C.L., Jay, M., Blurton, P., Burkamp, F., Clarkson, R. and Bristow, L.J. (2000) Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT_{2C/2B} receptor antagonists: neurochemical and behavioural studies. *Neuropharmacology*, 39: 2318–2328.
- Ichikawa, J., Dai, J. and Meltzer, H.Y. (2001a) DOI, a 5-HT_{2A/2C} receptor agonist, attenuates clozapine-induced cortical dopamine release. *Brain Res.*, 907: 151–155.
- Ichikawa, J., Dai, J. and Meltzer, H.Y. (2005) Lithium differs from anticonvulsant mood stabilizers in prefrontal cortical and accumbal dopamine release: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 1049: 182–190.
- Ichikawa, J., Ishii, H., Bonaccorso, S., Fowler, W.L., O'Laughlin, I.A. and Meltzer, H.Y. (2001b) 5-HT_{2A} and D₂ receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.*, 76: 1521–1531.
- Ichikawa, J., Kuroki, T., Kitchen, M.T. and Meltzer, H.Y. (1995) R(+)-8-OH-DPAT, a 5-HT_{1A} receptor agonist, inhibits amphetamine-induced dopamine release in rat striatum and nucleus accumbens. *Eur. J. Pharmacol.*, 287: 179–184.
- Ichikawa, J., Li, Z., Dai, J. and Meltzer, H.Y. (2002) Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 956: 349–357.
- Ichikawa, J. and Meltzer, H.Y. (1992) Amperozide, a novel antipsychotic drug, inhibits the ability of D-amphetamine to increase dopamine release in vivo in rat striatum and nucleus accumbens. *J. Neurochem.*, 58: 2285–2291.
- Ichikawa, J. and Meltzer, H.Y. (1995a) DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. *Brain Res.*, 698: 204–208.
- Ichikawa, J. and Meltzer, H.Y. (1995b) Effect of antidepressants on striatal and accumbens extracellular dopamine levels. *Eur. J. Pharmacol.*, 281: 255–261.
- Ichikawa, J. and Meltzer, H.Y. (1999a) R(+)-8-OH-DPAT, a serotonin_{1A} receptor agonist, potentiated S(–)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. *J. Pharmacol. Exp. Ther.*, 291: 1227–1232.
- Ichikawa, J. and Meltzer, H.Y. (1999b) Valproate and carbamazepine increase prefrontal dopamine release by 5-HT_{1A} receptor activation. *Eur. J. Pharmacol.*, 380: R1–R3.
- Ichikawa, J. and Meltzer, H.Y. (2000) The effect of serotonin_{1A} receptors on antipsychotic drug-induced dopamine release in rat striatum and nucleus accumbens. *Brain Res.*, 858: 252–263.
- Imperato, A. and Angelucci, L. (1989) 5-HT₃ receptors control dopamine release in the nucleus accumbens of freely moving rats. *Neurosci. Lett.*, 101: 214–217.
- Invernizzi, R., Pozzi, L. and Samanin, R. (1995) Selective reduction of extracellular dopamine in the rat nucleus accumbens following chronic treatment with DAU6215, a 5-HT₃ receptor antagonist. *Neuropharmacology*, 34: 211–215.
- Invernizzi, R.W., Pierucci, M., Calcagno, E., Di Giovanni, G., Di Matteo, V., Benigno, A. and Esposito, E. (2007) Selective activation of 5-HT_{2C} receptors stimulates gaba-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience*, 144: 1523–1535.
- Invernizzi, R.W., Sacchetti, G., Parini, S., Acconcia, S. and Samanin, R. (2003) Flibanterin, a potential antidepressant drug, lowers 5-HT and raises dopamine and noradrenaline in the rat prefrontal cortex dialysate: role of 5-HT_{1A} receptors. *Br. J. Pharmacol.*, 139: 1281–1288.
- Iyer, R.N. and Bradberry, C.W. (1996) Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 277: 40–47.
- Jenck, F., Bös, J., Wichmann, J., Stadler, H., Martin, J.R. and Moreau, J.L. (1998) The role of 5-HT_{2C} receptors in affective disorders. *Exp. Opin. Invest. Drugs*, 7: 1587–1599.

- Jenck, F., Moreau, J.L., Mutel, V. and Martin, J.R. (1994) Brain 5-HT_{1C} receptors and antidepressants. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 18: 563–574.
- Jenck, F., Moreau, J.L., Mutel, V., Martin, J.R. and Haefely, W.E. (1993) Evidence for a role of 5-HT_{1C} receptors in the antiserotonergic properties of some antidepressant drugs. *Eur. J. Pharmacol.*, 231: 223–229.
- Jiang, L.H., Ashby, C.R.J., Kasser, R.J. and Wang, R.Y. (1990) The effect of intraventricular administration of the 5-HT₃ receptor agonist 2-methylserotonin on the release of dopamine in the nucleus accumbens: an in vivo chronocoulometric study. *Brain Res.*, 513: 156–160.
- Johnson, S.W., Mercuri, N.B. and North, R.A. (1992) 5-Hydroxytryptamine_{1B} receptors block the GABA_B synaptic potential in rat dopamine neurons. *J. Neurosci.*, 12: 2000–2006.
- Jones, B.J. and Blackburn, T.P. (2002) The medical benefit of 5-HT research. *Pharmacol. Biochem. Behav.*, 71: 555–568.
- Kalivas, P.W. (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Rev.*, 18: 75–113.
- Kankaanpää, A., Lillsunde, P., Ruotsalainen, M., Ahtee, L. and Seppälä, T. (1996) 5-HT₃ receptor antagonist MDL 72222 dose-dependently attenuates cocaine- and amphetamine-induced elevations of extracellular dopamine in the nucleus accumbens and the dorsal striatum. *Pharmacol. Toxicol.*, 78: 317–321.
- Kankaanpää, A., Meririnne, E. and Seppälä, T. (2002) 5-HT₃ receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. *Psychopharmacology*, 159: 341–350.
- Kehne, J.H., Ketteler, H.J., McCloskey, T.C., Sullivan, C.K., Dudley, M.W. and Schmidt, C.J. (1996) Effects of the selective 5-HT_{2A} receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. *Neuropsychopharmacology*, 15: 116–124.
- Kennett, G.A. (1993) 5-HT_{1C} receptors and their therapeutic relevance. *Curr. Opin. Invest. Drugs*, 2: 317–362.
- Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D.R., Baxter, G.S., Forbes, I.T., Ham, P. and Blackburn, T.P. (1996) In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5HT_{2B} receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.*, 117: 427–434.
- Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, V., Avenel, K.J., Stean, T., Upton, N., Bromidge, S., Forbes, I.T., Brown, A.M., Middlemiss, D.N. and Blackburn, T.P. (1997) SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacology*, 36: 609–620.
- Kilpatrick, G., Jones, B. and Tyers, M. (1987) Identification and distribution of 5-HT₃ receptors in rat brain using radioligand binding. *Nature*, 330: 746–748.
- Kiyatkin, E.A. (1995) Functional significance of mesolimbic dopamine. *Neurosci. Biobehav. Rev.*, 19: 573–598.
- Knobelman, D.A., Kung, H.F. and Lucky, I. (2000) Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT_{1A} and 5-HT_{1B} receptors. *J. Pharmacol. Exp. Ther.*, 292: 1111–1117.
- Koch, S., Perry, K.W. and Bymaster, F.P. (2004) Brain region and dose effects of an olanzapine/fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology*, 46: 232–242.
- Koob, G.F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.*, 13: 177–184.
- Kurata, K., Ashby, C.R., Oberlander, R., Tanii, Y., Kurakchi, M., Rini, N.J. and Strecker, R.E. (1996) The characterization of the effect of locally applied *n*-methylquipazine, a 5-HT₃ receptor agonist, on extracellular dopamine levels in the anterior medial prefrontal cortex in the rat: an in vivo microdialysis study. *Synapse*, 24: 313–321.
- Kuroki, T., Kawahara, T., Yonezawa, Y. and Tashiro, N. (1999a) Effects of the serotonin_{2A/2C} receptor agonist and antagonist on phencyclidine-induced dopamine release in rat medial prefrontal cortex. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 23: 1259–1275.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (1999b) Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J. Pharmacol. Exp. Ther.*, 288: 774–781.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (2003) 5-HT_{2A} receptor stimulation by DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Brain Res.*, 972: 216–221.
- Lacroix, L.P., Dawson, L.A., Hagan, J.J. and Heidbreder, C.A. (2004) 5-HT₆ receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. *Synapse*, 51: 158–164.
- Laporte, A., Koscielniak, T., Ponchant, M., Verge, D., Hamon, M. and Gozlan, H. (1992) Quantitative autoradiographic mapping of 5-HT₃ receptors in the rat CNS using [¹²⁵I]iodozacopride and [³H]zacopride as radioligands. *Synapse*, 10: 271–281.
- Le Moal, M. and Simon, H. (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol. Rev.*, 71: 155–234.
- Leyse, J.E., Gommeren, W., Van Gompel, P., Wynants, J., Janssen, P.F. and Laduron, P.M. (1985) Receptor-binding properties in vitro and in vivo of ritanserin: a very potent and long acting serotonin-S₂ antagonist. *Mol. Pharmacol.*, 27: 600–611.
- Li, Z., Huang, M., Prus, A.J., Dai, J. and Meltzer, H.Y. (2007) 5-HT₆ receptor antagonist SB-399885 potentiates haloperidol and risperidone-induced dopamine efflux in the medial prefrontal cortex or hippocampus. *Brain Res.*, 1134: 70–78.
- Li, Z., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2004) Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. *Eur. J. Pharmacol.*, 493: 75–83.
- Li, Z., Ichikawa, J., Huang, M., Prus, A.J., Dai, J. and Meltzer, H.Y. (2005) ACP-103, a 5-HT_{2A/2C} inverse agonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Psychopharmacology*, 183: 144–153.

- Li, Z., Ichikawa, J. and Meltzer, H.Y. (2003) A comparison of the effects of loxapine with ziprasidone and thioridazine on the release of dopamine and acetylcholine in the prefrontal cortex and nucleus accumbens. *Psychopharmacology*, 167: 315–323.
- Liégeois, J.F., Ichikawa, J. and Meltzer, H.Y. (2002) 5-HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res.*, 947: 157–165.
- Liu, S., Bubar, M.J., Lanfranco, M.F., Hillman, G.R. and Cunningham, K.A. (2007) Serotonin_{2C} receptor localization in gaba neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*, 146: 1677–1688.
- Lôo, H., Hale, A. and D'Haenen, H. (2002) Determination of the dose of agomelatine, a melatonergic agonist and selective 5-HT_{2C} antagonist, in the treatment of major depressive disorder: a placebo-controlled dose range study. *Int. Clin. Psychopharmacol.*, 17: 239–247.
- Lucas, G., De Deurwaerdère, P., Caccia, S. and Spampinato, U. (2000) The effect of serotonergic agents on haloperidol-induced striatal dopamine release in vivo: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology*, 39: 1053–1063.
- Lucas, G., Di Matteo, V., De Deurwaerdère, P., Porras, G., Martin-Ruiz, R., Artigas, F., Esposito, E. and Spampinato, U. (2001) Neurochemical and electrophysiological evidence that 5-HT₄ receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal, dopaminergic function. *Eur. J. Neurosci.*, 13: 889–898.
- Lucas, G. and Spampinato, U. (2000) Role of striatal serotonin_{2A} and serotonin_{2C} receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. *J. Neurochem.*, 74: 693–701.
- Maj, J. and Moryl, E. (1992) Effects of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. *J. Neural Transm.: Gen. Sec.*, 88: 143–156.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Jr., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1h]indole] a novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.
- Martin, J.R., Bös, M., Jenck, F., Moreau, J.L., Mutel, V., Sleight, A.J., Wichmann, J., Andrews, J.S., Berendsen, H.H.G., Broekkamp, C.L.E., Ruigt, G.S.F., Köhler, C. and van Delft, A.M.L. (1998) 5-HT_{2C} agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.*, 286: 913–924.
- Matsumoto, M., Togashi, H., Mori, K., Ueno, K.I., Miyamoto, A. and Yoshioka, M. (1999) Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. *Eur. J. Pharmacol.*, 383: 39–48.
- Maurel-Remy, S., Bervoets, K. and Millan, M.J. (1995) Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100,907 in rats reflects antagonism of 5-HT_{2A} receptors. *Eur. J. Pharmacol.*, 280: R9–R11.
- McCreary, A.C. and Cunningham, K.A. (1999) Effects of the 5-HT_{2C/2B} antagonist SB 206553 on hyperactivity induced by cocaine. *Neuropsychopharmacology*, 20: 556–564.
- McCreary, A.C., Glennon, J.C., Ashby, C.R., Meltzer, H.Y., Li, Z., Reinders, J.H., Hesselink, M.B., Long, S.K., Herremans, A.H., van Stuivenberg, H., Feenstra, R.W. and Kruse, C.G. (2007) SLV313 (1-(2,3-dihydro-benzo [1,4] dioxin-5-yl)-4-[5-(4-fluoro-phenyl)-pyridin-3-ylmethyl]-piperazine monohydrochloride): a novel dopamine D₂ receptor antagonist and 5-HT_{1A} receptor agonist potential antipsychotic drug. *Neuropsychopharmacology*, 32: 78–94.
- McMahon, L.R. and Cunningham, K.A. (2001) Antagonism of 5-hydroxytryptamine_{2A} receptors attenuates the behavioral effects of cocaine in rats. *J. Pharmacol. Exp. Ther.*, 297: 357–363.
- McNeish, C.S., Svingos, A.L., Hitzemann, R. and Strecker, R.E. (1993) The 5-HT₃ antagonist zacopride attenuates cocaine-induced increases in extracellular dopamine in rat nucleus accumbens. *Pharmacol. Biochem. Behav.*, 45: 759–763.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21: 106S–115S.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159–1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D₁, D₂ and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251: 238–246.
- Meltzer, H.Y. and Nash, J.F. (1991) VII. Effects of antipsychotic drugs on serotonin receptors. *Pharmacol. Rev.*, 43: 587–604.
- Miller, C.H., Fleischacker, W.W., Ehrmann, H. and Kane, J.M. (1990) Treatment of neuroleptic induced akathisia with the 5-HT₂ antagonist ritanserin. *Psychopharm. Bull.*, 26: 373–376.
- Millan, M.J., Dekene, A. and Gobert, A. (1998a) Serotonin (5-HT)_{2C} receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT release in the frontal cortex in vivo. *Neuropharmacology*, 37: 953–955.
- Millan, M.J., Gobert, A., Lejeune, F., Dekeyne, A., Newman-Tancredi, A., Pasteau, V., Rivet, J.-M. and Cussac, D. (2003) The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine_{2C} receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J. Pharmacol. Exp. Ther.*, 306: 954–964.
- Millan, M.J., Gobert, A., Newman-Tancredi, A., Audinot, V., Lejeune, F., Rivet, J.M., Cussac, D., Nicolas, J.P., Muller, O. and Lavielle, G. (1998b) S16924 ((R)-2-[1-[2-(2,3-dihydro-benzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolidin-3-yl]-1-(4-fluorophenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)_{1A} agonist properties: I. Receptorial

- and neurochemical profile in comparison with clozapine and haloperidol. *J. Pharmacol. Exp. Ther.*, 286: 1341–1355.
- Millan, M.J., Gobert, A., Rivet, J.M., Adhumeau-Auclair, A., Cussac, D., Newman-Tancredi, A., Dekeyne, A., Nicolas, J.P. and Lejeune, F. (2000) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of α_2 -adrenergic and serotonin_{2C} receptors: a comparison with citalopram. *Eur. J. Neurosci.*, 12: 1079–1095.
- Moghaddam, B. and Bunney, B.S. (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. *J. Neurochem.*, 54: 1755–1760.
- Molineaux, S.M., Jessell, T.M., Axel, R. and Julius, D. (1989) 5-HT_{1C} receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.*, 86: 6793–6797.
- Monsma, F.J., Jr., Shen, Y., Ward, R.P., Hamblin, M.W. and Sibley, D.R. (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.*, 43: 320–327.
- Morales, M., Battenberg, E. and Bloom, F.E. (1998) Distribution of neurons expressing immunoreactivity for the 5-HT₃ receptor subtype in the rat brain and spinal cord. *J. Comp. Neurol.*, 385: 385–401.
- Morales, M., Battenberg, E., de Lecea, L. and Bloom, F.E. (1996) The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. *Brain Res.*, 731: 199–202.
- Morales, M. and Bloom, F.E. (1997) The 5-HT₃ receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J. Neurosci.*, 17: 3157–3167.
- Moreau, J.L., Bös, M., Jenck, F., Martin, J.R., Mortas, P. and Wichmann, J. (1996) 5-HT_{2C} receptor agonists exhibit antidepressant-like properties in the anhedonia model of depression in rats. *Eur. Neuropsychopharmacol.*, 6: 169–175.
- Moreau, J.L., Bourson, A., Jenck, F., Martin, J.R. and Mortas, P. (1994) Curative effects of the atypical antidepressant mianserin in the chronic mild stress-induced anhedonia model of depression. *J. Psychiatry Neurosci.*, 19: 51–56.
- Moreau, J.L., Jenck, F., Martin, J.R., Perrin, S. and Haefely, W.E. (1993) Effect of repeated mild stress and two antidepressant treatments on the behavioral response to 5-HT_{1C} receptor activation in rats. *Psychopharmacology*, 110: 140–144.
- Morikawa, H., Manzoni, O.J., Crabbe, J.C. and Williams, J.T. (2000) Regulation of central synaptic transmission by 5-HT_{1B} receptors. *Mol. Pharmacol.*, 58: 1271–1278.
- Morrow, B.A., Elsworth, J.D., Zito, C. and Roth, R.H. (1999) Biochemical and behavioral anxiolytic-like effects of *R*(+)-HA-966 at the level of the ventral tegmental area in rats. *Psychopharmacology*, 143: 227–234.
- Moukhes, H., Bosler, O., Bolam, J.P., Vallée, A., Umbriaco, D., Geffard, M. and Doucet, G. (1997) Quantitative and morphometric data indicate precise cellular interactions between serotonin terminals and postsynaptic targets in rat substantia nigra. *Neuroscience*, 76: 1159–1171.
- Nakayama, K., Sakurai, T. and Katsu, H. (2004) Mirtazapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. *Brain Res. Bull.*, 63: 237–241.
- Navailles, S., De Deurwaerdère, P.D., Porras, G. and Spampinato, U. (2004) In vivo evidence that 5-HT_{2C} receptor antagonist but not agonist modulates cocaine-induced dopamine outflow in the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 29: 319–326.
- Navailles, S., De Deurwaerdère, P.D. and Spampinato, U. (2006a) Clozapine and haloperidol differentially alter the constitutive activity of central serotonin_{2C} receptors in vivo. *Biol. Psychiatry*, 59: 568–575.
- Navailles, S., Moison, D., Cunningham, K.A. and Spampinato, U. (2008) Differential regulation of the mesoaccumbens dopamine circuit by serotonin_{2C} receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. *Neuropsychopharmacology*, 33: 237–246.
- Navailles, S., Moison, D., Ryczko, D. and Spampinato, U. (2006b) Region-dependent regulation of mesoaccumbens dopamine neurons in vivo by the constitutive activity of central serotonin_{2C} receptors. *J. Neurochem.*, 99: 1311–1319.
- Nelson, D.L. (2004) 5-HT₅ receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3: 53–58.
- Newton, R.A. and Elliott, J.M. (1997) Mianserin-induced down-regulation of human 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptors stably expressed in the human neuroblastoma cell line SH-SY5Y. *J. Neurochem.*, 69: 1031–1038.
- Nicholson, S.L. and Brotchie, J.M. (2002) 5-Hydroxytryptamine (5-HT, serotonin) and Parkinson's disease — opportunities for novel therapeutics to reduce the problems of levodopa therapy. *Eur. J. Neurol.*, 9: 1–6.
- Nocjar, C., Roth, B.L. and Pehek, E.A. (2002) Localization of 5-HT_{2A} receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, 111: 163–176.
- Nomikos, G.G., Iurlo, M., Andersson, J.L., Kimura, K. and Svensson, T.H. (1994) Systemic administration of amperozide, a new atypical antipsychotic drug, preferentially increases dopamine release in the rat medial prefrontal cortex. *Psychopharmacology*, 115: 147–156.
- O'Dell, L. and Parsons, L. (2004) Serotonin_{1B} receptors in the ventral tegmental area modulate cocaine-induced increases in nucleus accumbens dopamine levels. *J. Pharmacol. Exp. Ther.*, 311: 711–719.
- O'Neill, M.F., Heron-Maxwell, C.L. and Shaw, G. (1999) 5-HT₂ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. *Pharmacol. Biochem. Behav.*, 63: 237–243.
- Pandi-Perumal, S.R., Srinivasan, V., Cardinali, D.P. and Monti, M.J. (2006) Could agomelatine be the ideal antidepressant? *Expert Rev. Neurother.*, 6: 1595–1608.
- Parsons, L., Koob, G.F. and Weiss, F. (1999) RU24969, a 5-HT_{1B/1A} receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. *Synapse*, 32: 132–135.

- Pasqualetti, M., Ori, M., Castagna, M., Marazziti, D., Cassano, G.B. and Nardi, I. (1999) Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. *Neuroscience*, 92: 601–611.
- Patel, S., Roberts, J., Moorman, J. and Reavill, C. (1995) Localization of serotonin-4 receptors in the striatonigral pathway in rat brain. *Neuroscience*, 69: 1159–1167.
- Pehek, E.A. (1996) Local infusion of the serotonin antagonists ritanserin or ICS 205,930 increases in vivo dopamine release in the rat medial prefrontal cortex. *Synapse*, 24: 12–18.
- Pehek, E.A. and Bi, Y. (1997) Ritanserin administration potentiates amphetamine-stimulated dopamine release in the rat prefrontal cortex. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 21: 671–682.
- Pehek, E.A., McFarlane, H.G., Maguschak, K., Price, B. and Pluto, C.P. (2001) M100,907, a selective 5-HT_{2A} antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res.*, 888: 51–59.
- Pehek, E.A., Nocjar, C., Roth, B.L., Byrd, T.A. and Mabrouk, O.S. (2006) Evidence for the preferential involvement of 5-HT_{2A} serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. *Neuropsychopharmacology*, 31: 265–277.
- Pei, Q., Zetterstrom, T., Leslie, R. and Grahame-Smith, D. (1993) 5-HT₃ receptor antagonists inhibit morphine-induced stimulation of mesolimbic dopamine release and function in the rat. *Eur. J. Pharmacol.*, 230: 63–68.
- Pierucci, M., Di Matteo, V. and Esposito, E. (2004) Stimulation of serotonin_{2C} receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.*, 309: 109–118.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12: 440–453.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.*, 23: 163–178.
- Porras, G., De Deurwaerdère, P., Moison, D. and Spampinato, U. (2003) Conditional involvement of striatal serotonin₃ receptors in the control of in vivo dopamine outflow in the rat striatum. *Eur. J. Neurosci.*, 17: 771–781.
- Porras, G., Di Matteo, V., De Deurwaerdère, P., Esposito, E. and Spampinato, U. (2002a) Central serotonin₄ receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: in vivo studies with morphine, amphetamine and cocaine. *Neuropharmacology*, 43: 1099–1109.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdère, P., Caccia, S., Esposito, E. and Spampinato, U. (2002b) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26: 311–324.
- Pozzi, L., Acconcia, S., Ceglia, I., Invernizzi, R.W. and Samanin, R. (2002) Stimulation of 5-hydroxytryptamine (5-HT_{2C}) receptors in the ventrosegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J. Neurochem.*, 82: 93–100.
- Pozzi, L., Trabace, L., Invernizzi, R. and Samanin, R. (1995) Intrastriatal GR 113808, a selective 5-HT₄ receptor antagonist, attenuates morphine-stimulated dopamine release in the rat striatum. *Brain Res.*, 692: 265–268.
- Pranzatelli, M.R., Murthy, J.N. and Taylor, P.T. (1993) Novel regulation of 5-HT_{1C} receptors: down-regulation induced both by 5-HT_{1C/2} receptor agonists and antagonists. *Eur. J. Pharmacol.*, 244: 1–5.
- Prinssen, E.P.M., Koek, W. and Kleven, M.S. (2000) The effects of antipsychotics with 5-HT_{2C} receptor affinity in behavioral assays selective for 5-HT_{2C} receptor antagonist properties of compounds. *Eur. J. Pharmacol.*, 388: 57–67.
- Prisco, S. and Esposito, E. (1995) Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. *Br. J. Pharmacol.*, 116: 1923–1931.
- Prisco, S., Pagannone, S. and Esposito, E. (1994) Serotonin–dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. *J. Pharmacol. Exp. Ther.*, 271: 83–90.
- Puglisi-Allegra, S., Imperato, A., Angelucci, L. and Cabib, S. (1991) Acute stress induces time-dependent responses in dopamine mesolimbic system. *Brain Res.*, 554: 217–222.
- Radja, F., Descarrier, L., Dewar, K.M. and Reader, T.A. (1993) Serotonin 5-HT₁ and 5-HT₂ receptors in adult rat brain after destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. *Brain Res.*, 606: 273–285.
- Rasmusson, A.M., Goldstein, L.E., Deutch, A.Y., Bunney, B.S. and Roth, R.H. (1994) 5-HT_{1A} agonist \pm 8-OH-DPAT modulates basal and stress-induced changes in medial prefrontal cortical dopamine. *Synapse*, 18: 218–224.
- Rausser, L., Savage, J.E., Meltzer, H.Y. and Roth, B.L. (2001) Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine_{2C} receptor. *J. Pharmacol. Exp. Ther.*, 299: 83–89.
- Reavill, C., Kettle, A., Holland, V., Riley, G. and Blackburn, T.P. (1999) Attenuation of haloperidol-induced catalepsy by a 5-HT_{2C} receptor antagonist. *Br. J. Pharmacol.*, 126: 572–574.
- Roberts, J.C., Reavill, C., East, S.Z., Harrison, P.J., Patel, S., Routledge, C. and Leslie, R.A. (2002) The distribution of 5-HT₆ receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT₆ receptor antagonist [¹²⁵I]SB-258585. *Brain Res.*, 934: 49–57.
- Rocha, B.A., Goulding, E.H., O'Dell, L.E., Mead, A.N., Coufal, N.G., Parsons, L.H. and Tecott, L.H. (2002) Enhanced locomotor, reinforcing, and neurochemical effects of cocaine in serotonin 5-hydroxytryptamine 2C receptor mutant mice. *J. Neurosci.*, 22: 10039–10045.
- Rodriguez, M.C., Obeso, J.A. and Olanow, C.W. (1998) Subthalamic nucleus-mediated excitotoxicity in Parkinson's disease: a target for neuroprotection. *Ann. Neurol.*, 44(Suppl.): S175–S188.
- Rollema, H., Lu, Y., Schmidt, A.W., Sprouse, J. and Zorn, S.H. (2000) 5-HT_{1A} receptor activation contributes to

- ziprasidone-induced dopamine release in rat prefrontal cortex. *Biol. Psychiatry*, 48: 229–237.
- Roth, B.L., Craig, S.C., Choudhary, M.S., Uluer, A., Monsma, F.J., Jr., Shen, Y., Meltzer, H.Y. and Sibley, D.R. (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.*, 268: 1403–1410.
- Roth, R.H. and Elsworth, J.D. (1995) Biochemical pharmacology of midbrain dopamine neurons. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 227–243.
- Roth, B.L., Roland, D., Ciaranello, D. and Meltzer, H.Y. (1992) Binding of typical and atypical antipsychotic agents to transiently expressed 5-HT_{1C} receptors. *J. Pharmacol. Exp. Ther.*, 260: 1361–1365.
- Roth, R.H., Wolf, M.E. and Deutch, A.Y. (1987) Neurochemistry of midbrain dopamine systems. In: Meltzer H.Y. (Ed.), *Psychopharmacology: The Third Generation of Progress*. Raven Press, New York, pp. 81–94.
- Ruat, M., Traiffort, E., Arrang, J.M., Tardivel-Lacombe, J., Diaz, J., Leurs, R. and Schwartz, J.C. (1993a) A novel rat serotonin 5-HT₆ receptor: molecular cloning, localization, and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.*, 193: 268–276.
- Ruat, M., Traiffort, E., Leurs, R., Tardive-lacombe, J., Diaz, J., Arrang, J.M. and Schwartz, J.C. (1993b) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT₇) activating cAMP formation. *Proc. Natl. Acad. Sci. U.S.A.*, 90: 8547–8551.
- Sakaue, M., Somboonthum, P., Nishihara, B., Koyama, Y., Hashimoto, H., Baba, A. and Matsuda, T. (2000) Post-synaptic 5-hydroxytryptamine_{1A} receptor activation increases in vivo dopamine release in rat prefrontal cortex. *Br. J. Pharmacol.*, 129: 1028–1034.
- Sampson, D., Muscat, R. and Willner, P. (1991) Reversal of antidepressant action by dopamine antagonists in an animal model of depression. *Psychopharmacology*, 104: 491–495.
- Santana, N., Bortolozzi, A., Serrats, J., Mengod, G. and Artigas, F. (2004) Expression of serotonin_{1A} and serotonin_{2A} receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex*, 14: 1100–1109.
- Sawaguchi, T. and Goldman-Rakic, P.S. (1994) The role of D₁ dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.*, 71: 515–528.
- Schmidt, C.J. and Fadayel, G.M. (1996) Regional effects of MK-801 on dopamine release: effects of competitive NMDA or 5-HT_{2A} receptor blockade. *J. Pharmacol. Exp. Ther.*, 277: 1541–1549.
- Schmidt, C.J., Fadayel, G.M., Sullivan, C.K. and Taylor, V.L. (1992) 5-HT₂ receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur. J. Pharmacol.*, 223: 65–74.
- Schmidt, C.J., Sorensen, S.M., Kehne, J.H., Carr, A.A. and Palfreyman, M.G. (1995) The role of 5-HT_{2A} receptors in antipsychotic activity. *Life Sci.*, 25: 2209–2222.
- Schmidt, C.J., Sullivan, C.K. and Fadayel, G.M. (1994) Blockade of striatal 5-hydroxytryptamine₂ receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.*, 62: 1382–1389.
- Schotte, A., de Bruyckere, K., Janssen, P.F. and Leysen, J.E. (1989) Receptor occupancy by ritanserin and risperidone measured using ex vivo autoradiography. *Brain Res.*, 500: 295–301.
- Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., Van Gompel, P., Lesage, A.S., De Loore, K. and Leysen, J.E. (1996) Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology*, 124: 57–73.
- Seiden, L.S., Sabol, K.E. and Ricaurte, G.A. (1993) Amphetamine: effects on catecholamine systems and behavior. *Annu. Rev. Pharmacol. Toxicol.*, 32: 639–677.
- Serretti, A., Artioli, P. and De Ronchi, D. (2004) The 5-HT_{2C} receptor as a target for mood disorders. *Expert Opin. Ther. Targets*, 8: 1–9.
- Sharma, A., Punhani, T. and Fone, K.C.F. (1997) Distribution of the 5-hydroxytryptamine_{2C} receptor protein in adult rat brain and spinal cord determined using a receptor-directed antibody: effect of 5,7-dihydroxytryptamine. *Synapse*, 27: 45–56.
- Shen, Y., Monsma, F.J., Jr., Metcalf, M.A., Jose, P.A., Hamblin, M.W. and Sibley, D.R. (1993) Molecular cloning and expression of a 5-hydroxytryptamine₇ serotonin receptor subtype. *J. Biol. Chem.*, 268: 18200–18204.
- Shilliam, C.S. and Dawson, L.A. (2005) The effect of clozapine on extracellular dopamine levels in the shell subregion of the rat nucleus accumbens is reversed following chronic administration: comparison with a selective 5-HT_{2C} receptor antagonist. *Neuropsychopharmacology*, 30: 372–380.
- Spanagel, R. and Weiss, F. (1999) The dopamine hypothesis of reward: past and current status. *Trends Neurosci.*, 22: 521–527.
- Steinbush, H.W.M. (1984) Serotonin-immunoreactive neurons and their projections in the CNS. In: Björklund A., Hökfelt T. and Kuhar M.J. (Eds.), *Handbook of Chemical Neuroanatomy: Classical Transmitter Receptors in the CNS, Part II*. Elsevier Science Publishers B.V., Amsterdam, pp. 68–125.
- Steffensen, S.C., Svingos, A.L., Pickel, V.M. and Henriksen, S.J. (1998) Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J. Neurosci.*, 18: 8003–8015.
- Steward, L.J., Ge, J., Stowe, R.L., Brown, D.C., Bufton, R.K., Stokes, P.R.A. and Barnes, N.M. (1996) Ability of 5-HT₄ receptor ligands to modulate rat striatal dopamine release in vitro and in vivo. *Br. J. Pharmacol.*, 117: 55–62.
- Svensson, T.H., Mathe, J.M., Andersson, J.L., Nomikos, G.G., Hildebrand, B.E. and Marcus, M. (1995) Mode of action of atypical neuroleptics in relation to the phencyclidine model of schizophrenia: role of 5-HT₂ receptor and alpha

- 1-adrenoceptor antagonism. *J. Clin. Psychopharmacol.*, 15: 11S–18S.
- Svensson, T.H., Nomikos, G.G. and Andersson, J.L. (1993) Modulation of dopaminergic neurotransmission by 5-HT₂ antagonism. In: Vanhouette P.M., Saxena P.R., Paoletti R., Brunello N. and Jackson A.S. (Eds.), *Serotonin: From Cell Biology to Pharmacology and Therapeutics*. Kluwer Academic Publishers, Dordrecht, pp. 263–270.
- Takeda, H., Tsuji, M., Ikoshi, H., Yamada, T., Masuya, J., Imori, M. and Matsumiya, T. (2005) Effects of a 5-HT₇ receptor antagonist DR4004 on the exploratory behavior in a novel environment and on brain monoamine dynamics in mice. *Eur. J. Pharmacol.*, 518: 30–39.
- Tanda, G., Bassareo, V. and Di Chiara, G. (1996) Mianserin markedly and selectively increases extracellular dopamine in the prefrontal cortex as compared to the nucleus accumbens of the rat. *Psychopharmacology*, 123: 127–130.
- Tanda, G., Carboni, E., Frau, R. and Di Chiara, G. (1994) Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology*, 155: 285–288.
- Tanda, G., Frau, R. and Di Chiara, G. (1995) Local 5-HT₃ receptors mediate fluoxetine but not desipramine-induced increase of extracellular dopamine in the prefrontal cortex. *Psychopharmacology*, 119: 15–19.
- Taylor, S.G. and Routledge, C. (1996) Lack of effect of systemically administered 5-HT₄ agonists on dopamine levels measured from the nucleus accumbens and striatum: an in vivo microdialysis study in freely-moving rats. *Br. J. Pharmacol.*, 118(Suppl.): p. 326P.
- Thorré, K., Ebinger, G. and Michotte, Y. (1998) 5-HT₄ receptor involvement in the serotonin-enhanced dopamine efflux from the substantia nigra of the freely moving rat: a microdialysis study. *Brain Res.*, 796: 117–124.
- Tomkins, D.M., Joharchi, N., Tampakeras, M., Martin, J.R., Wichmann, J. and Higgins, G.A. (2002) An investigation of the role of 5-HT_{2C} receptors in modifying ethanol self-administration behaviour. *Pharmacol. Biochem. Behav.*, 71: 735–744.
- Ullmer, C., Engels, P., Abdel'Al, S. and Lubbert, H. (1996) Distribution of 5-HT₄ receptor mRNA in the rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 354: 210–212.
- Ungerstedt, U. (1991) Microdialysis — principles and applications for studies in animals and man. *J. Intern. Med.*, 230: 365–373.
- Utter, A.A. and Basso, M.A. (2008) The basal ganglia: an overview of circuits and function. *Neurosci. Biobehav. Rev.*, 32: 333–342.
- Van Bockstaele, E.J., Biswas, A. and Pickel, V.M. (1993) Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res.*, 624: 188–198.
- Van Bockstaele, E.J., Cestari, D.M. and Pickel, V.M. (1994) Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. *Brain Res.*, 647: 307–322.
- Van Bockstaele, E.J. and Pickel, V.M. (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.*, 682: 215–221.
- Van der Kooy, D. and Attori, T. (1980) Dorsal raphe cells with collateral projections to the caudate-putamen and substantia nigra: a fluorescent retrograde double labeling study in the rat. *Brain Res.*, 186: 1–7.
- Van Oekelen, D., Luyten, W.H. and Leysen, J.E. (2003) 5-HT_{2A} and 5-HT_{2C} receptors and their atypical regulation properties. *Life Sci.*, 72: 2429–2449.
- Vilario, M.T., Cortes, R., Gerald, C., Branchek, T.A., Palacios, J.M. and Mengod, G. (1996) Localization of 5-HT₄ receptor mRNA in rat brain by in situ hybridization histochemistry. *Mol. Brain Res.*, 43: 356–360.
- Vilario, M.T., Cortes, R. and Mengod, G. (2005) Serotonin 5-HT₄ receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. *J. Comp. Neurol.*, 484: 418–439.
- Volonté, M., Monferini, E., Cerutti, M., Fodritto, F. and Borsini, F. (1997) BIMG 80, a novel potential antipsychotic drug: evidence for multi-receptor actions and preferential release of dopamine in prefrontal cortex. *J. Neurochem.*, 69: 182–190.
- Ward, R.P. and Dorsa, D.M. (1996) Colocalization of serotonin receptor subtypes 5-HT_{2A}, 5-HT_{2C} and 5-HT₆ with neuropeptides in rat striatum. *J. Comp. Neurol.*, 370: 405–414.
- Wędzony, K., Maćkowiak, M., Golembiowska, K. and Fijał, K. (1996) Apsapirone enhances the dopamine outflow via 5-HT_{1A} receptors in the rat prefrontal cortex. *Eur. J. Pharmacol.*, 305: 73–78.
- Westerink, B.H.C., Kawahara, Y., De Boer, P., Geels, C., De Vries, J.B., Wikström, H.V., Van Kalker, A., Van Vliet, B., Kruse, C.G. and Long, S.K. (2001) Antipsychotic drugs classified by their effects on the release of dopamine and noradrenaline in the prefrontal cortex and striatum. *Eur. J. Pharmacol.*, 412: 127–138.
- White, F.J. (1996) Synaptic regulation of mesocorticolimbic dopamine neurons. *Annu. Rev. Neurosci.*, 19: 405–436.
- Willins, D.L. and Meltzer, H.Y. (1998) Serotonin 5-HT_{2C} agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. *Brain Res.*, 781: 291–299.
- Willner, P. (1995) Animal models of depression: validity and applications. In: Gessa G., Fratta W., Pani L. and Serra G. (Eds.), *Depression and Mania: From Neurobiology to Treatment*. Raven Press, New York, pp. 19–41.
- Wood, M.D., Reavill, C., Trail, B., Wilson, A., Stean, T., Kennett, G.A., Lightowler, S., Blackburn, T.P., Thomas, D., Gager, T.L., Riley, G., Holland, V., Bromidge, S.M., Forbes, I.T. and Middlemiss, D.N. (2001) SB-243213; a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. *Neuropharmacology*, 41: 186–199.
- Wozniak, K.M., Pert, A. and Linnoila, M. (1990) Antagonism of 5-HT₃ receptors attenuates the effects of ethanol on extracellular dopamine. *Eur. J. Pharmacol.*, 187: 287–289.
- Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M. and Jennes, L. (1995) Comparative localization of serotonin_{1A,1C}, and ₂ receptor subtype mRNAs in rat brain. *J. Comp. Neurol.*, 351: 357–373.

- Yamamoto, B.K., Nash, J.F. and Gudelsky, G.A. (1995) Modulation of methylenedioxymethamphetamine-induced striatal dopamine release by the interaction between serotonin and gamma-aminobutyric acid in the substantia nigra. *J. Pharmacol. Exp. Ther.*, 273: 1063–1070.
- Yan, Q.S. and Yan, S.E. (2001) Activation of 5-HT_{1B/1D} receptors in the mesolimbic dopamine system increases dopamine release from the nucleus accumbens: a microdialysis study. *Eur. J. Pharmacol.*, 418: 55–64.
- Yan, Q.S., Zheng, S.Z., Feng, M.J. and Yan, S.E. (2005) Involvement of 5-HT_{1B} receptors within the ventral tegmental area in ethanol-induced increases in mesolimbic dopaminergic transmission. *Brain Res.*, 1060: 126–137.
- Yan, Q.S., Zheng, S.Z. and Yan, S.E. (2004) Involvement of 5-HT_{1B} receptors within the ventral tegmental area in regulation of mesolimbic dopaminergic neuronal activity via GABA mechanisms: a study with dual-probe microdialysis. *Brain Res.*, 1021: 82–91.
- Yoshimoto, K., Yayama, K., Sorimachi, Y., Tani, J., Ogata, M., Nishimura, A., Yoshida, T., Ueda, S. and Komura, S. (1996) Possibility of 5-HT₃ receptor involvement in alcohol dependence: a microdialysis study of nucleus accumbens dopamine and serotonin release in rats with chronic alcohol consumption. *Alcohol Clin. Exp. Res.*, 20: 311A–319A.
- Yoshino, T., Nisijima, K., Katoh, S., Yui, K. and Nakamura, M. (2002) Tansospirone potentiates the fluoxetine-induced increases in extracellular dopamine via 5-HT_{1A} receptors in the rat frontal cortex. *Neurochem. Int.*, 40: 355–360.
- Yoshino, T., Nisijima, K., Shioda, K., Yui, K. and Katoh, S. (2004) Perospirone, a novel atypical antipsychotic drug, potentiates fluoxetine-induced increases in dopamine levels via multireceptor actions in the rat medial prefrontal cortex. *Neurosci. Lett.*, 364(1): 16–21.
- Zhang, W., Perry, K.W., Wong, D.T., Potts, B.D., Bao, J., Tollefson, G.D. and Bymaster, F.P. (2000) Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology*, 23: 250–262.
- Zocchi, A., Fabbri, D. and Heidbreder, C.A. (2005) Aripiprazole increases dopamine but not noradrenaline and serotonin levels in the mouse prefrontal cortex. *Neurosci. Lett.*, 387: 157–161.
- Zupancic, M. and Guilleminault, C. (2006) Agomelatine: a preliminary review of a new antidepressant. *CNS Drugs*, 20: 981–992.

CHAPTER 3

Serotonin–dopamine interaction: electrophysiological evidence

Giuseppe Di Giovanni¹, Vincenzo Di Matteo², Massimo Pierucci² and Ennio Esposito^{2,*}

¹*Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana “G. Pagano”, Università di Palermo,
Corso Tuköry 129, 90134 Palermo, Italy*

²*Istituto di Ricerche Farmacologiche “Mario Negri”, Consorzio “Mario Negri”, Via Nazionale,
66030 Santa Maria Imbaro, Chieti, Italy*

Abstract: In this review, the most relevant data regarding serotonin (5-hydroxytryptamine, 5-HT)/dopamine (DA) interaction in the brain, as studied by both in vivo and in vitro electrophysiological methods, are reported and discussed. The bulk of neuroanatomical data available clearly indicate that DA-containing neurons in the brain receive a prominent innervation from 5-HT originating in the raphe nuclei of the brainstem. Furthermore, this modulation seems to be reciprocal; DA neurons innervate the raphe nuclei and exert a tonic excitatory effect on them. Compelling electrophysiological data show that 5-HT can exert complex effects on the electrical activity of midbrain DA neurons mediated by the various receptor subtypes. The main control seems to be inhibitory, this effect being more marked in the ventral tegmental area (VTA) as compared to the substantia nigra pars compacta (SNc). In spite of a direct effect of 5-HT by its receptors located on DA cells, 5-HT can modulate their activity indirectly, modifying γ -amino-*n*-butyric acid (GABA)-ergic and glutamatergic input to the VTA and SNc. Although 5-HT/DA interaction in the brain has been extensively studied, much work remains to be done to clarify this issue. The recent development of subtype-selective ligands for 5-HT receptors will not only allow a detailed understanding of this interaction but also lead to development of new treatment strategies, appropriate for those neuropsychiatric disorders in which an alteration of the 5-HT/DA balance is supposed.

Keywords: serotonin; serotonin receptors; dopamine; electrophysiology; substantia nigra; ventral tegmental area

Introduction

This chapter is devoted to the analysis and discussion of the most relevant data regarding serotonin (5-hydroxytryptamine, 5-HT)/dopamine (DA) interaction in the brain, as studied by

electrophysiological methods. Both in vivo and in vitro findings will be reviewed, although emphasis will be given to data obtained by in vivo extracellular single-cell recordings. The bulk of neuroanatomical data available clearly indicate that DA-containing neurons in the brain receive a prominent innervation from 5-HT originating in the raphe nuclei of the brainstem (Fig. 1). Thus, the detailed knowledge of this neuroanatomical wiring is an obvious prerequisite for the

*Corresponding author. Tel.: +39 0872 570274;
Fax: +39 0872 570416; E-mail: esposito@negrissud.it

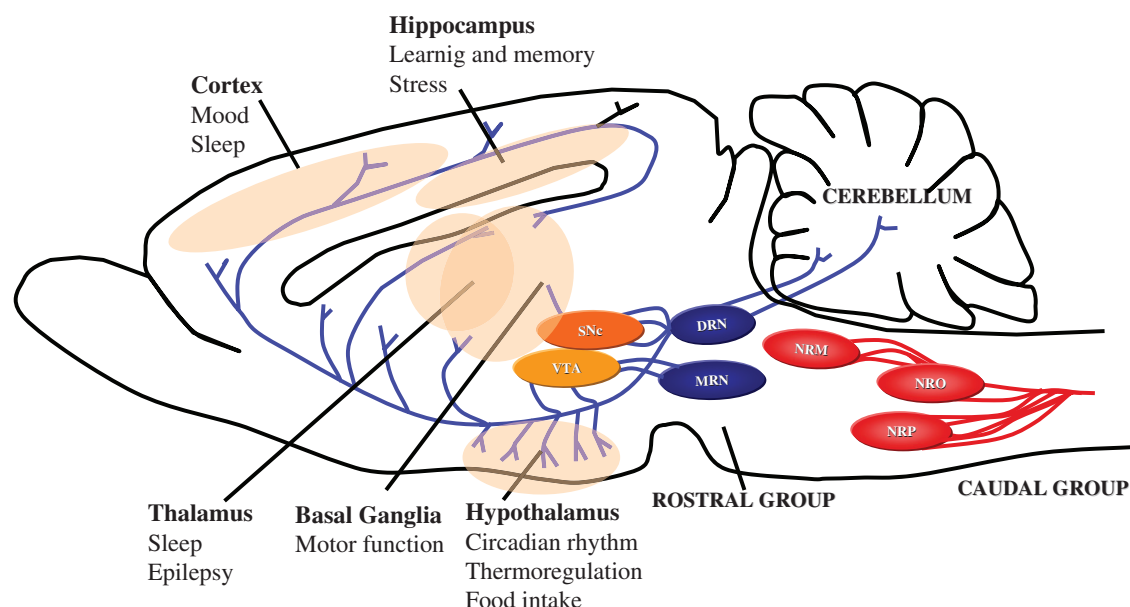


Fig. 1. Midsagittal view of the rat brain with serotonin-immunoreactive cell bodies. The blue and red ovals encompass the two major subdivisions of the brain serotonergic system. The major functions of 5-HT and the brain areas where they occur are also shown. Abbreviations: DRN, dorsal raphe nucleus; MRN, medial raphe nucleus; NRM, nucleus raphe magnus; NRO, nucleus raphe obscurus; SNc, substantia nigra pars compacta; VTA, ventral tegmental area. (See Color Plate 3.1 in the color plate section).

understanding of the functional interaction of 5-HT and DA systems, including the electrophysiological findings.

The presence of 5-HT-containing nerve fibres in the substantia nigra (SN) and the ventral tegmental area (VTA) was first demonstrated by Fuxe (1965) using formaldehyde-induced fluorescence histochemistry. Subsequent studies have confirmed and extended these findings, showing also that 5-HT fibres coming from the dorsal raphe nucleus (DRN) were distributed significantly to the SN and the VTA (Fibiger and Miller, 1977; Azmitia and Segal, 1978; Steinbusch, 1981; Hervé et al., 1987; Mori et al., 1987; Wirtshafter et al., 1987; Lavoie and Parent, 1990; Van Bockstaele et al., 1994; Van Bockstaele and Pickel, 1994; Moukhles et al., 1997; Vertes and Linley, 2007), and to the main projection areas of the nigro-striatal and the mesolimbic DA-ergic system, such as the corpus striatum, the globus pallidus, the nucleus accumbens, amygdala, olfactory tubercle and the frontal cortex (Azmitia and Segal, 1978; Steinbusch, 1981; Lavoie and Parent, 1990; Vertes and Linley, 2007). In contrast, 5-HT fibres arising from medial raphe

nucleus (MRN) innervate the VTA but not the SN, although the concentration of 5-HT terminals in this area is less dense as compared to the SN (Fibiger and Miller, 1977; Azmitia and Segal, 1978; Steinbusch, 1981; Mori et al., 1987; Wirtshafter et al., 1987; Lavoie and Parent, 1990; Vertes and Linley, 2007). In fact, it is noteworthy that the SN receives the densest 5-HT innervation of the brain in several animal species, including rats (Palkovits et al., 1974; Saavedra, 1977; Moukhles et al., 1997), monkeys (Shannak and Ornykiewicz, 1980; Lavoie and Parent, 1990) and humans (Fahn et al., 1971; Mackay et al., 1978). Other investigations revealed the raphe-nigral projection arises mainly from the DRN and terminates principally in the substantia nigra pars reticulata (SNr) (Azmitia and Segal, 1978; Mori et al., 1987; Lavoie and Parent, 1990; Corvaja et al., 1993; Moukhles et al., 1997). Thus, it has been reported that 5-HT neurons innervate both DA and non-DA neurons in the VTA (Hervé et al., 1987; Van Bockstaele et al., 1994), with as many as 50% of [^3H]5-HT forming synaptic contacts in this area (Hervé et al., 1987). Of these 5-HT terminals, about

32% formed synaptic junctions with perikarya or dendrites containing tyrosine hydroxylase (TH) immunoreactivity (i.e. DA neurons) (Van Bockstaele et al., 1994).

Quantitative autoradiographic mapping of 5-HT in the rat brain showed the presence of 5-HT receptor subtypes on both nigro-striatal and mesocorticolimbic DA-ergic systems (Pazos et al., 1985; Pazos and Palacios, 1985). Thus, by using tritiated 5-HT ligands, it was found that 5-HT₁ receptors were present in the SN and, in particular, in the SNr (which contains high concentrations of 5-HT_{1B} receptors), the corpus striatum and several areas of the mesocorticolimbic system such as the VTA, the nucleus accumbens, the amygdala, the olfactory tubercle and the frontal cortex (Pazos and Palacios, 1985). Also, 5-HT_{2A} receptors (which at that time were simply named 5-HT₂) are distributed in most DA-ergic areas of the brain, including the substantia nigra pars compacta (SNc), the VTA, the striatum, the nucleus accumbens, the amygdala, the olfactory tubercle, the entorhinal cortex and the frontal cortex (Pazos et al., 1985). Autoradiographic mapping studies also showed the presence of 5-HT_{2C} receptors (which at that time were defined 5-HT_{1C}, and were labelled by tritiated 5-HT and mesulergine) in several brain regions, including nuclei of origin and terminal areas of central DA-ergic systems, such as the SNc, the VTA, the striatum, the

nucleus accumbens, the entorhinal cortex and the frontal cortex (Pazos and Palacios, 1985). Subsequent autoradiographic studies confirmed the presence of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors in several DA-ergic areas of the human brain (Hoyer et al., 1986; Pazos et al., 1987; Marazziti et al., 1999). In addition, relatively high densities of 5-HT₃ and 5-HT₄ receptor subtypes have been detected in terminal areas of the mesolimbic DA-ergic system, whereas low or undetectable levels of 5-HT₃ receptors are present in the VTA, or the nigro-striatal DA system (Barnes et al., 1990; Kilpatrick et al., 1996) (Table 1).

A number of studies, using in situ hybridization histochemistry and immunohistochemical techniques, have shown the presence of 5-HT_{2A} and 5-HT_{2C} mRNAs receptor proteins localized on neuronal components of the nigro-striatal and mesocorticolimbic DA-ergic systems of rodents (Molineaux et al., 1989; Mengod et al., 1990a, b; Pompeiano et al., 1994; Wright et al., 1995; Ward and Dorsa, 1996; Eberle-Wang et al., 1997; Clemett et al., 2000; Doherty and Pickel, 2000; Ikemoto et al., 2000; Bubar and Cunningham, 2007; Liu et al., 2007) and humans (Abramowski et al., 1995; Pasqualetti et al., 1999), whereas 5-HT_{2B} receptor proteins were undetectable on these neurons (Duxon et al., 1997). It is important to note that double-label in situ hybridization in the SN reveals co-expression of 5-HT_{2C} receptor mRNA with

Table 1. The anatomical distribution of serotonin receptors in the CNS

Receptor subtype	Receptor distribution
5-HT _{1A}	Raphe nuclei (somatodendritic autoreceptors), limbic system (amygdala, hippocampus, septum)
5-HT _{1B}	Basal ganglia (terminal autoreceptors)
5-HT _{1D}	Raphe nuclei
5-HT _{1E}	Caudate putamen, entorhinal cortex, parietal cortex, thalamus, hypothalamus
5-HT _{1F}	Dorsal raphe, hippocampus, cortex, striatum, thalamus, hypothalamus
5-HT _{2A}	Cortex, basal ganglia, spinal cord
5-HT _{2B}	Cerebellum, lateral septum, hypothalamus
5-HT _{2C}	Dorsal raphe, choroid plexus, medulla, pons, striatum, nucleus accumbens, hippocampus, hypothalamus, ventrotergental area, substantia nigra, spinal cord
5-HT _{3A/3B/3C}	Caudate putamen, hippocampus, entorhinal cortex, frontal cortex, cingulate cortex and dorsal horn ganglia
5-HT ₄	Striatum, brainstem, thalamus, hippocampus, substantia nigra
5-HT _{5A/5B}	Hippocampus, cortex, cerebellum, habenula, spinal cord
5-HT ₆	Caudate-putamen, olfactory tubercle, nucleus accumbens, cortex, hippocampus
5-HT ₇	Hippocampus, hypothalamus, thalamus, raphe nuclei

Table 2. Comparison of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}

Receptor	Signal transduction	Receptor distribution	Splice variants
5-HT _{2A}	PLC, c-fos, ion flux, PKC activation	Cortex > basal ganglia > hippocampus	None identified yet
5-HT _{2B}	Ras activation, Ca ²⁺ flux	Small amounts in various brain areas	None identified yet
5-HT _{2C}	PLC, PLA ₂ , PLD	Choroid plexus > hippocampus > striatum	Yes

glutamic acid decarboxylase (GAD) but not with TH mRNA, indicating that 5-HT_{2C} receptors are restricted to γ -amino-*n*-butyric acid (GABA)-ergic neurons, both in adult rats (Eberle-Wang et al., 1997) and in humans (Pasqualetti et al., 1999). However, it was recently found that in the VTA, 5-HT_{2C} receptor protein is expressed on both DA and GABA-containing neurons (Bubar and Cunningham, 2007). Similarly, 5-HT_{2A} receptors are localized on both DA (about 20–40% of the population, according to different authors) and non-DA neurons in the VTA (Cornea-Hebert et al., 1999; Doherty and Pickel, 2000; Ikemoto et al., 2000; Nocjar et al., 2002), although in this case the neurochemical nature of non-DA neurons was not established. The presence of both 5-HT_{2A} and 5-HT_{2C} receptors has also been detected in neurons containing GABA, acetylcholine, enkephalin, substance P or dynorphin, in various terminal regions of the nigro-striatal and meso-corticolimbic DA system, such as the striatum (Ward and Dorsa, 1996), the nucleus accumbens, the lateral amygdala, the pyriform cortex and the medial prefrontal cortex (mPFC) (Morilak et al., 1993; Liu et al., 2007).

The 5-HT_{2A} and 5-HT_{2C} receptors display 50% overall amino acid sequence homology and >80% sequence homology within the transmembrane domain regions (Boess and Martin, 1994; Martin and Humphrey, 1994). Likewise, the 5-HT_{2A} and 5-HT_{2C} receptors are both coupled to G_{αq/11} and activate the downstream effector phospholipase C (PLC), thus stimulating the hydrolysis of membrane phospholipids and the production of inositol-1-4,5-triphosphate and diacylglycerol, leading to increased intracellular Ca²⁺ (Hoyer et al., 2002). Moreover, both receptors couple with G_{αq/11} to activate phospholipase A₂ (PLA₂), independent of PLC activation (Felder et al., 1990). Despite these similarities in signalling

mechanisms, subtle differences between 5-HT_{2A} and 5-HT_{2C} signalling cascades also exist (Table 2). In addition, agonists have been shown to activate signalling pathways differentially and to couple to their effectors in an agonist-independent manner (constitutive activity), although this differs with regard to receptor and effectors (Berg et al., 2005). For example, the 5-HT_{2C} receptors express greater constitutive activity towards PLC, but the 5-HT_{2C} receptor has relatively weak constitutive activity towards PLA₂ (determined by arachidonic acid release) (Berg et al., 2005).

Functional interactions between dopamine and serotonin neurons: electrophysiological evidence

Dopamine modulation of serotonergic neurons activity

It is noteworthy that midbrain DA nuclei also control the activity of 5-HT neurons; thus, the control is reciprocal in nature. Indeed, a dense direct projection from the SNc to the DRN by retrograde tracer technique has been characterized (Sakai et al., 1977). These results have been extended successively using injection of tritiated leucine and proline into the VTA and SNc-labelled fibres, confirming an overlapping descending pathway to the DRN (Beckstead et al., 1979). The DA-ergic innervation of the DRN has been confirmed by more recent studies using a combination of fluorescent retrograde tracing and fluorescence histochemistry anti-DA antibodies (Decavel et al., 1987; Kalén et al., 1988; Peyron et al., 1995; Kitahama et al., 2000). In addition, it has been shown that 5-HT neurons express D₂-like (D₂ and D₃) DA receptors (Mansour et al., 1990; Suzuki et al., 1998). These anatomical results suggest that DA input to the DRN may play a

critical role in the regulation of the function of DRN 5-HT neurons. There is consistent evidence regarding the DA-ergic regulation of DRN 5-HT neurons. Infusion of the DA agonist apomorphine in the rat DRN stimulates the firing rate of 5-HT neurons and the local release of 5-HT, while these effects are partially prevented by the selective D₂ receptor antagonist raclopride (Ferre and Artigas, 1993; Martin-Ruiz et al., 2001).

Consistent with these findings is the recent evidence from in vitro electrophysiological studies that activation of DA₂ receptors, presumably located on DRN 5-HT neurons, induces a membrane slow depolarization and thereby increases their excitability (Haj-Dahmane, 2001; Aman et al., 2007). In contrast to its conventional effect (i.e. inhibition of adenylyl cyclase and membrane hyperpolarization), in DRN neurons, the D₂ signal involves a G-protein-dependent process, activation of PLC and a non-selective cationic conductance (Aman et al., 2007).

DA-ergic lesion on 5-HT neuron activity

The effect of selective lesion of DA neurons elicited by 6-hydroxydopamine (6-OHDA) has been recently investigated (Chu et al., 2004; Zhang et al., 2007; Guiard et al., 2008) following the study by Svensson et al. (1975). The loss of DA innervation is capable of altering the firing properties of the DRN serotonergic neurons, although the results have been contradictory. In fact, some studies (Svensson et al., 1975; Chu et al., 2004; Zhang et al., 2007) have shown that in 6-OHDA-lesioned rats, 5-HT neurons do not modify their discharge or fire at a higher frequency (+70%) and preferentially display burst firing activity. On the other hand, Guiard et al. (2008) showed that the discharge rate of 5-HT neurons was instead reduced by 60%, indicating that DA input exerts a tonic excitatory effect on 5-HT neurons in the intact brain. The DA lesion did not change the number of DRN 5-HT neurons recorded per track, although the DRN 5-HT neuronal firing was markedly attenuated in lesioned rats. However, previous electrophysiological data indicate that conditions that decrease the firing activity of DRN 5-HT neurons by about 50% do not necessarily

modify the number of neurons found per track (Blair et al., 1986). The lack of DA might cause inhibition of 5-HT because of the loss of excitatory DA tone on D₂ receptors (Haj-Dahmane, 2001). It is unlikely that the excitatory effects of DA involved a local release of glutamate, since the pharmacological inactivation of ionotropic and metabotropic glutamate receptors did not prevent the DA-induced depolarization of DRN 5-HT membrane (Haj-Dahmane, 2001).

Serotonin modulation of DA neuronal activity

In vivo recordings from neurochemically identified DA neurons were made possible by the pioneering work of Bunney et al. (1973a, b) who were the first to record the electrical activity of single DA neurons in the SNc and the VTA of anaesthetized rats. The neurochemical identity of those neurons as DA-ergic was confirmed by a great number of subsequent studies (Aghajanian and Bunney, 1977; Grace and Bunney, 1980, 1983a, b, 1984, 1986; Wang, 1981; Chiodo and Bunney, 1985). It is now well established that the electrophysiological characteristics of extracellularly recorded DA-containing neurons, in both the SNc and the VTA, are the following: (1) biphasic or triphasic action potentials (with a positive first phase), (2) action potential durations between 2 and 5 ms and peak-to-peak amplitudes of 0.5–1.5 mV, (3) single-spike firing pattern or burst firing with each burst containing two to eight spikes of decreasing amplitude and increasing duration, and (4) almost always display a notch on the initial rising phase of the action potential, which represents the initial segment-somatodendritic (IS-SD) break (Bunney et al., 1973a, b; Aghajanian and Bunney, 1977; Grace and Bunney, 1980, 1983a, b, 1984, 1986; Wang, 1981; Chiodo and Bunney, 1985; Chiodo, 1988) (Fig. 2). It is obvious that the precise neurochemical identification of DA neurons is an essential prerequisite when studying the interaction between the serotonergic and the DA-ergic systems. Indeed, the first electrophysiological study aimed at investigating the effect of 5-HT on DA electrical activity was performed on neurochemically identified DA neurons in the SNc (Aghajanian and Bunney, 1974). Microiontophoretic

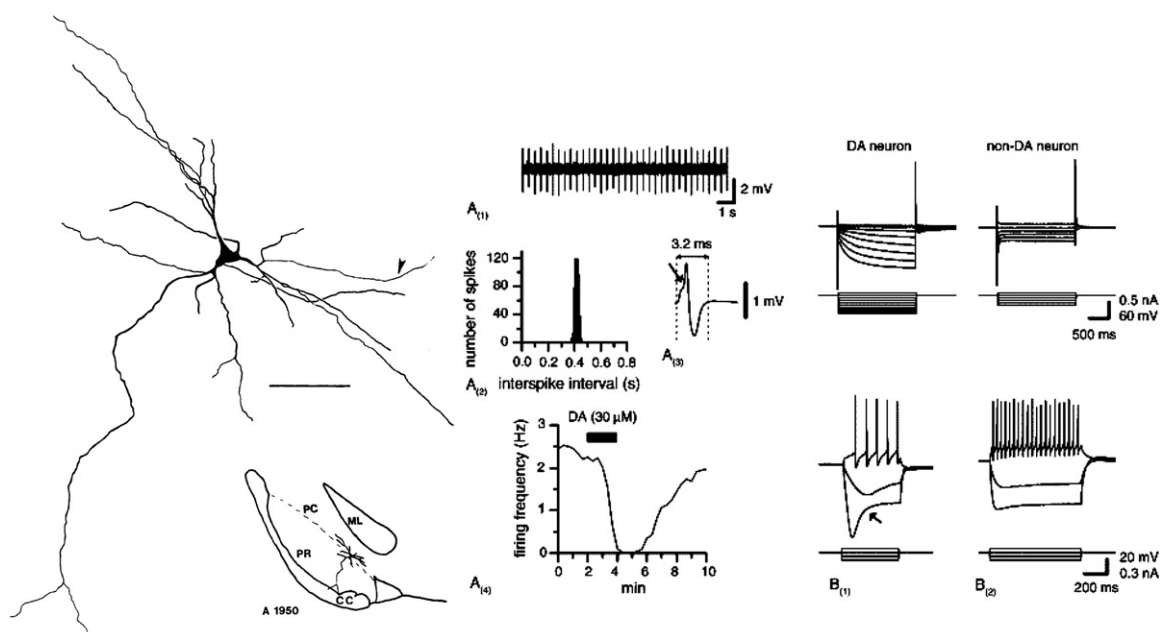


Fig. 2. Characteristics of a DA-ergic neuron. Left panel: Reconstruction of an electrophysiologically identified nigro-striatal DA-ergic neuron intracellularly labelled with HRP following *in vivo* intracellular recording. The inset at lower right shows the position of the neuron with respect to the boundaries of the substantia nigra. Right panel: Identification of SNc neurons in extracellular (A) and whole-cell patch-clamp (B) recordings. (A) Example of extracellular recording from a DA neuron. $A_{(1)}$: Spontaneous firing. $A_{(2)}$: Interval histogram demonstrating regularity of spontaneous firing. $A_{(3)}$: Shape of individual action potentials (average of 16 consecutive potentials). Note inflection on the rising phase of the action potential (arrow). $A_{(4)}$: Inhibition of firing after bath application of dopamine ($30 \mu\text{M}$). Source: Electrophysiologically nigral dopaminergic neurons intracellularly labelled with HRP: microscopic analysis. (B) Example of whole-cell patch-clamp recording. $B_{(1)}$: DA neuron. From top: I_h current induced by hyperpolarizing voltage commands (voltage-clamp), and voltage responses to step current pulses recorded in current-clamp. Note depolarizing sag of the membrane potential (arrow) evoked by hyperpolarizing current pulses. $B_{(2)}$: Non-DA neuron. From above: currents induced by voltage steps (voltage-clamp), and responses to step current pulses (current-clamp). Adapted with permission from Berretta et al. (2005) and Tepper et al. (1987). Source: Acute effects of 6-hydroxydopamine on dopaminergic neurons of the rat substantia nigra pars compacta *in vitro*.

application of 5-HT was found to exert only weak inhibitory effects on the firing rate of DA neurons (Aghajanian and Bunney, 1974). However, most of the early electrophysiological studies on this subject were carried out recording the electrical activity of neurons indiscriminately, in both the SNc and the SNr, and without using the criteria for the neurochemical identification of the neurons recorded (Dray et al., 1976, 1978; Fibiger and Miller, 1977).

Serotonergic lesion on DA neuronal activity

The *i.c.v.* injection of the toxin 5,7-dihydroxytryptamine (5,7-DHT) produces a robust and selective decrease in brain 5-HT levels (Prisco and

Esposito, 1995; Prisco et al., 1994; Di Mascio et al., 1999; Guiard et al., 2008). In fact, the toxin treatment resulted in significant depletions of 5-HT in the corpus striatum (-66.5%) and hippocampus (-90%) (Di Mascio et al., 1999) and in the frontal cortex (-87%) (Guiard et al., 2008). Conversely, 5,7-DHT lesions did not cause any change in the basal interspike interval (ISI) standard characteristics (firing rate and bursting activity) either in the VTA (Prisco and Esposito, 1995; Prisco et al., 1994) or in the SNc (Kelland et al., 1990). The basal firing rate of VTA DA-ergic neurons in 5-HT-depleted rats was higher than in control animals, but this difference was not statistically significant (Prisco and Esposito, 1995; Prisco et al., 1994). Also, depletion of brain 5-HT

had little effect on the basal activity of nigrostriatal DA neurons. However, both 5-HT depleters *para*-chlorophenylalanine (PCPA) and 5,7-DHT produced small but significant reductions in the conduction velocity of these cells (Kelland et al., 1990). Minabe et al. (1996) showed that depletion of brain 5-HT by administration of PCPA produced a significant decrease in the number of spontaneously active DA cells in both the SNc (52%) and the VTA (63%) areas, compared to controls. The burst firing analysis indicated that there was a significant increase in the mean ISI, with a decrease in both the burst firing pattern and the number of bursts of SNc and VTA DA neurons in PCPA-treated animals. The intravenous (i.v.) administration of 5-hydroxytryptophan and the peripheral aromatic acid decarboxylase inhibitor benserazide, which restores 5-HT content, reversed both the decrease in the number of spontaneously active SNc and VTA DA neurons and the decrease in the percentage of VTA DA neurons exhibiting a bursting pattern.

It was noticeable that, although we did not reveal any modification of DA conventional firing characteristics on 5-HT-depleted animals (Prisco et al., 1994), in successive experiments, using the non-linear prediction method combined with Gaussian-scaled surrogate, we showed a decrease in chaos of the electrical activity of VTA DA neurons, extracellularly recorded in vivo lesioned with 5,7-DHT (Di Mascio et al., 1999). The term chaos is here intended to describe a highly erratic, yet deterministic behaviour. Moreover, in the control (unlesioned) group, a positive correlation was found between the functional operator (Ψ), equivalent to the density power spectrum of the signals and the ISIs, and the chaos content measure by non-linear prediction S score, a relation that was lost in the lesioned group (Di Mascio et al., 1999). We suggested that the decreased chaotic behaviour of DA-ergic neurons in lesioned rats, which have a decreased serotonergic tone, might represent a preclinical phenomenon of the onset of psychiatric disorders in humans. In fact, chaotic behaviour, more than periodic or stochastic behaviour, makes biological systems more capable of responding to different stimuli without causing damage.

Recently, Guiard et al. (2008) showed contrasting results with the above findings, supporting the hypothetical inhibitory 5-HT tone on DA neurons. In their experimental conditions 5,7-DHT lesion leads to a 36% increase in the discharge rate of VTA DA neurons in rats. This enhancement of DA neuronal activity resulted from a higher number of bursts and spikes per burst (Guiard et al., 2008).

Serotonin reuptake inhibitors on DA neuron activity

In accordance with a putative inhibitory effect of the 5-HT input on DA neurons is the finding that the indirect serotonergic agonists such as selective serotonin reuptake inhibitors (SSRIs) are capable of inhibiting the basal activity of DA neurons in the VTA. Thus, acute administration of fluoxetine causes a dose-dependent inhibition of the firing rate of VTA DA-ergic neurons, but it does not affect the activity of DA cells in the SNc (Prisco and Esposito, 1995). A similar effect, though less pronounced, has been observed with citalopram (Prisco and Esposito, 1995). Acute injection of fluvoxamine, paroxetine and sertraline caused a dose-dependent inhibition of some VTA DA neurons but it did not affect the basal firing rate of other DA cells. These findings provide further support that electrophysiological interactions between DA and 5-HT are subtle, but important, with differences between the SNc and the VTA that could explain (in part) the effectiveness and lower propensity to induce side-effects of the newer atypical antipsychotic drugs. In addition, we showed that acute injection of fluvoxamine, paroxetine and sertraline caused a dose-dependent inhibition only of some VTA DA neurons, but did not affect the basal firing rate of other DA cells (Prisco and Esposito, 1995). A fast Fourier transformation-based analysis of the basal activity of VTA DA neurons showed a positive correlation between the value of a functional operator Ψ and the degree of SSRI-induced inhibition of VTA DA cells (Di Mascio and Esposito, 1997). The VTA neurons not affected by SSRIs' treatment showed a more regular behaviour of the ISI functions corresponding to lower values detected by the functional operator Ψ , whereas the neurons

inhibited by SSRIs showed a less regular behaviour of ISI functions corresponding to higher Ψ values, detected by the same functional operator. Fluvoxamine, paroxetine and sertraline also caused a dose-dependent increase of the percentage of spikes occurring in bursts in neurons (low values of Ψ), in some cells that were not affected. We suggested that this difference in density power spectrum could reflect the asymmetry of serotonergic input to the VTA DA neurons, and the differential effects of SSRIs on these neurons might depend on the characteristics of their basal firing mode (Di Mascio and Esposito, 1997).

Unexpected results have been provided by Sekine et al. (2007) who examined the effect of the administration of fluoxetine, citalopram and paroxetine on the number of spontaneously active DA neurons in the SNc, using the cells-per-track technique in in vivo extracellular recording in rats. Apparently, systemic administration of some SSRIs increases the number of midbrain DA neurons with differential effects on VTA. Only the acute treatment of fluoxetine was able to increase significantly the number of spontaneously active SNc and VTA DA neurons, while chronic treatment with fluoxetine, citalopram and paroxetine increased selectively the number of VTA spontaneously active neurons (Sekine et al., 2007). The mechanism by which the SSRIs alter the number of spontaneously active DA neurons is, citing the same authors, 'unknown'. Overall, this study lacks crucial information that is usually obtained with these cells-per-track studies, i.e. firing rate and burst firing analysis of the recorded neurons. This evidence on this subject would have been useful.

Notwithstanding, the most accredited effect of the synaptic increase of 5-HT in midbrain DA-ergic nuclei is an inhibitory response. We have proposed that fluoxetine inhibits the mesolimbic DA pathway by enhancing the extracellular level of 5-HT, which would act through 5-HT_{2C} receptors (Prisco and Esposito, 1995). In fact, both mesulergine, an unselective 5-HT_{2A/2B/2C} receptor antagonist (Boess and Martin, 1994), and the destruction of 5-HT neurons by the neurotoxin 5,7-DHT prevent the fluoxetine-induced inhibition of VTA DA cells (Prisco and Esposito, 1995). We also demonstrated that fluoxetine-induced

inhibition of DA neurons in the VTA was no longer observed after chronic treatment (21 days) with this drug. Interestingly, mCPP, a mixed 5-HT_{2A/2B/2C} receptor agonist (Hoyer et al., 1994; Barnes and Sharp, 1999), inhibited the firing activity receptor agonist (Hoyer et al., 1994; Barnes and Sharp, 1999) and inhibited the firing activity of VTA DA neurons in control animals but not in those chronically treated with fluoxetine (Prisco and Esposito, 1995). We suggested that 5-HT_{2C} receptors might be down-regulated after repeated fluoxetine administration. Consistent with this hypothesis is the evidence that chronic treatment with sertraline and citalopram, two SSRIs, induces tolerance to the hypolocomotor effect of mCPP (Maj and Moryl, 1992). This hyposensitivity of 5-HT_{2C} receptors might be a key step for the achievement of an antidepressant effect. Indeed, it is possible to argue that the acute inhibitory effect of fluoxetine on the mesolimbic DA system would mask its clinical efficacy in the early stage of treatment. This masking effect would disappear when the hyposensitivity of 5-HT_{2C} receptors occurs. A series of studies carried out in our laboratory have shown that acute administration of SSRIs such as paroxetine, sertraline and fluvoxamine causes a slight but significant decrease in the basal firing rate of VTA DA-ergic neurons (Di Mascio and Esposito, 1997; Di Mascio et al., 1998). Therefore, it is conceivable that, similarly to fluoxetine, these three SSRIs could reduce mesocorticolimbic DA transmission by activating 5-HT_{2C} receptors, although this hypothesis awaits experimental confirmation. Interestingly, a correlation was found between the ED₅₀ of paroxetine, sertraline, fluvoxamine, citalopram and fluoxetine to suppress the basal activity of 5-HT neurons in the DRN and their maximal inhibition of VTA DA cell activity (Di Mascio et al., 1998). The involvement of 5-HT_{2C} receptors has been further highlighted by recent studies showing that their concurrent inactivation might be a novel strategy for enhancing the efficacy of SSRIs (Cremers et al., 2004, 2007; Boothman et al., 2006). These recent findings are consonant with our hypothesis that the down-regulation and/or blockade of 5-HT_{2C} receptors is a sine qua non of the therapeutic effect of SSRIs.

Electrical stimulation of the DRN and MRN on DA neuron activity

Microiontophoretic application of 5-HT caused mainly inhibition of neuronal activity, but excitation and biphasic effects were also observed (Dray et al., 1976, 1978). These early findings lead the authors of the studies to conclude that ‘... *the substantia nigra receives a direct monosynaptic inhibitory input from the DRN and MRN and those pathways use 5-HT as a neurotransmitter serving to tonically inhibit DA-ergic neurons ...*’ (Dray et al., 1976, 1978). Dray et al. (1976) also studied the effect of the stimulation of the MRN in the rat, revealing a predominant reduction of the activity of cells in the SN. This evidence was not confirmed by Fibiger and Miller (1977); in fact, single pulse stimulation of the MRN was relatively ineffective in their experimental conditions. Instead, they showed that a selective single stimulus delivered to the DRN was capable of causing complete cessation of spontaneously active nigral cells for periods ranging from 25 to 180 ms. The discrepancy between these two studies is probably due to the fact that Dray et al. (1976), by stimulating the MRN, could have activated serotonergic axons from the DRN, which descend in the vicinity of the MRN. Since PCPA blocked the inhibitory effects of DRN stimulation and did not significantly influence the spontaneous activity of cells in the SNc, it follows that the serotonergic projection from the DRN influences the DA-ergic cells in a phasic manner rather than exerting a tonic inhibitory action. Hence, it is only under certain circumstances that this inhibition is manifest.

Following these studies, Kelland et al. (1990, 1993) found that electrical stimulation of DRN, at low to moderate frequencies, inhibits all VTA DA neurons, as compared to inhibiting only SNc DA neurons with a basal activity below 4 Hz, which they defined as ‘slowly firing’. The inhibition of VTA neurons was significant at stimulation rates of 0.1 Hz and higher for slow cells, and at rates of 0.5 Hz and higher for fast cells. Maximal inhibition at 10.0 Hz was 85 ± 10 and $60 \pm 16\%$, respectively, and there was no significant difference between the slow and fast cell groups (Kelland et al., 1993). The inhibition in the SNc was seen only on slow

cells and was significant at stimulation rates of 0.5 Hz and higher, and reached a maximal inhibition of $66 \pm 7\%$ at 10.0 Hz (Kelland et al., 1990, 1993). This preferential influence of 5-HT on a subset of nigro-striatal DA neurons raises significant issues. These data suggest that slow nigro-striatal DA neurons may fire more slowly because they are under a tonic inhibitory influence by 5-HT. A likely correlate is that fast cells may also receive some preferential excitatory input, such as the excitatory input from the pedunculopontine tegmental nucleus, subthalamus and cortex. Thus, slow cells and fast cells, under basal conditions, may represent distinct populations of DA neurons with differential afferent regulation. In addition, these data help to explain why roughly half of the DA cells (the fast cells) might not respond to administration of 5-HT and related compounds.

Furthermore, Trent and Tepper (1991) reported that electrical stimulation of DRN decreased the SD excitability of DA neurons in the SNc expressed as reduction of the proportion of neostriatal-evoked antidromic responses. Conversely, the DRN stimulation did not significantly affect the basal firing rate and pattern of the SNc recorded, consistent with dissociation between the effects of raphe input on SD excitability and neuronal firing. The reduction in excitability is due, at least in part, to 5-HT release in the SNc, as indicated by the reversal of the effect by the 5-HT antagonist, metergoline, and by the abolition of the effect in rats depleted of 5-HT by PCPA treatment, and subsequent reinstatement of the effect by 5-HTP administration. Stimulation of the DRN activates a serotonergic raphe-nigral pathway synapsing on DA-ergic neurons. Synaptically released 5-HT activates a 5-HT receptor on the nigral somadendrite, which triggers a local dendritic release of DA, either by dendritic depolarization or by a direct action on a SD calcium conductance. The dendritically released DA in turn activates SD D_2 DA autoreceptors which hyperpolarize the SD membrane locally and result in conduction failure of the SD component of the antidromic response due either to the dendritic hyperpolarization or to the underlying increase in potassium conductance, or both (Trent and Tepper, 1991). The authors further supported the

thesis that, in vivo, the serotonergic input from the DRN acts in a phasic rather than tonic manner to regulate the presynaptic (DA-releasing) functions of nigral DA-ergic dendrites locally, without affecting neuronal excitability as a whole.

The latest evidence available is given by Gervais and Rouillard (2000). In vivo stimulation of 5-HT neurons in the DRN inhibited most of the recorded DA neurons in A9, while the majority of DA neurons in A10 was excited. Some cells exhibited an inhibition–excitation response, while in other DA neurons, the initial response was an excitation followed by an inhibition. In SNc, 56% of the DA cells recorded were initially inhibited and 31% of the DA cells were initially excited. In contrast, 63% of VTA DA cells were initially excited and 34% were initially inhibited. Depletion of endogenous 5-HT by 5,7-DHT and PCPA almost completely eliminated the inhibition–excitation response in both SNc and VTA DA cells, without changing the percentage of DA cells initially excited. Consequently, the proportion of DA neurons that were not affected by DRN stimulation increased after 5-HT depletion (from 13 to 60% in the SNc and from 6 to 31% in the VTA). In several DA cells, DRN stimulation caused important changes in firing rate and firing pattern. These data strongly confirm that the 5-HT input from the DRN is mainly inhibitory. It also suggests that 5-HT afferences modulate SNc and VTA DA neurons in an opposite manner. These results also suggest that non-5-HT inputs from DRN can also modulate mesencephalic DA neurons and have an excitatory role on the activity of these neurons (Gervais and Rouillard, 2000).

Serotonergic control of DA neuron activity: role of the different 5-HT receptor subtypes

Notwithstanding the methodological limitations of early electrophysiological studies, a number of subsequent, more rigorous investigations have confirmed that the prevalent action of the central serotonergic action on DA neuronal activity is inhibitory. The availability of selective 5-HT ligands has made it possible to single out the involvement of specific 5-HT receptor subtypes in this effect.

5-HT₁ family

As already mentioned, the majority of the available electrophysiological data on 5-HT–DA interaction have been obtained in vivo, by using extracellular recordings of single neurochemically identified DA neurons. One of the first studies which investigated the role of different 5-HT receptor subtypes on the electrical activity of midbrain DA was carried out by Sinton and Fallon (1988). These authors investigated the effects of ligands for 5-HT_{1A} and 5-HT_{1B} receptors on SNc DA neurons in chloral hydrate-anaesthetized rats. It was found that 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), potent and selective 5-HT_{1A} agonist, caused a slight excitation at relatively low doses, whereas it inhibited the basal activity of SNc DA neurons at much higher doses. These authors argued that the excitatory effect of 8-OH-DPAT resulted from disinhibition of a 5-HT input, whereas the inhibition of SNc DA neurons occurring at higher doses of this drug was due to its aspecific activation of D₂ DA receptors, in that it was blocked by haloperidol (Sinton and Fallon, 1988). Accordingly, the 5-HT_{1A} receptor agonists 8-OH-DPAT and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) preferentially increased the firing rate of slowly firing SNc DA neurons, but did not alter the responsiveness of these cells to quinpirole-induced inhibition of firing rate (Kelland et al., 1990).

Arborelius et al. (1993) failed to reveal any modification in the firing rate of SNc neurons, as only a slightly increased burst firing was induced by low dose of 8-OH-DPAT. Conversely, they found that systemic administration of 8-OH-DPAT caused a significant increase of firing rate and burst firing of the neurons within the VTA. The effect on firing rate of DA neurons was more pronounced in the parabrachial pigmentosus nucleus than in the paranigral nucleus, the two major subdivisions of VTA. We obtained similar results with 8-OH-DPAT that increased the firing rate of the majority (75%) of VTA DA neurons recorded, and stimulated their bursting activity (Prisco et al., 1994). Inasmuch as selective lesions of 5-HT neurons induced by the neurotoxin 5,7-DHT completely prevented the excitatory

effect of 8-OH-DPAT (Kelland et al., 1990; Prisco et al., 1994), the mechanism by which it causes its effect is probably indirect. 8-OH-DPAT, by suppressing the activity of raphe 5-HT neurons through stimulation of SD 5-HT_{1A} autoreceptors, decreases the inhibitory 5-HT input to the DA neurons. This conclusion was strengthened by the evidence that direct microiontophoretic application of 8-OH-PAT into the VTA did not cause any change in the basal activity of VTA DA neurons (Prisco et al., 1994). However, this latter finding was in contrast to that reported by Arborelius et al. (1993) showing that pressure ejection of 8-OH-DPAT into the VTA increased the activity of DA neurons. Lejeune and Millan (1998) provided compelling evidence that activation of 5-HT_{1A} receptors is associated with a reduction in forebrain serotonergic transmission and a reinforcement in DA-ergic input to the mPFC. The authors ruled out the involvement of presynaptic receptors, suggesting alternatively that the actions at postsynaptic 5-HT_{1A} receptors on VTA GABA-ergic interneurons were involved. This hypothesis has been confirmed by Doherty and Pickel (2001), using an immunocytochemical technique, revealing that VTA GABA-ergic interneurons express 5-HT_{1A} similarly distributed throughout the VTA. Activation of the inhibitory 5-HT_{1A} receptors can thus disinhibit, i.e. increase, SD DA release and may also enhance DA release in projection areas. More recently, the role of the postsynaptic 5-HT_{1A} receptor in the mPFC has been highlighted; these receptors seem to be deeply involved in the modulation of DA-ergic activity (Díaz-Mataix et al., 2005). In fact, the excitatory effect of the highly selective 5-HT_{1A} agonist BAY × 3702 (*R*-(−)-2-(4-[(chroman-2-ylmethyl)-amino]-butyl)-1,1-dioxo-benzo[*d*] isothiazolone hydrochloride) on VTA neurons was completely prevented by frontocortical transection (Díaz-Mataix et al., 2005). This indicates that 5-HT_{1A} activation may modulate the activity of corticotegmental, presumably glutamatergic, projections regulating DA neurons. Thus, 5-HT_{1A} modulation of DA function requires further clarification, especially since recent evidence indicates the presence of 5-HT_{1A} receptors on distinct DA cell subpopulations in the VTA parabrachial compared with VTA paranigral

nucleus, suggesting a greater role for VTA 5-HT_{1A} receptors in modulating the mesocortical DA system (Doherty and Pickel, 2001). This evidence is in agreement with the electrophysiological study by Arborelius et al. (1993).

Very few electrophysiological studies have instead investigated the role of 5-HT_{1B} receptors on DA function. The first evidence was given again by Sinton and Fallon (1988). They showed the unselective 5-HT_{1B} receptor agonists trifluoromethylphenylpiperazine (TFMPP) and CGS 12066B {7-trifluoromethyl-4-(4-methyl-1-piperazinyl)-pyrrolo[1,2-*a*]quinoxaline 1:2 maleate sal} markedly inhibited SNc DA neuron activity at relatively low doses (Sinton and Fallon, 1988). It was argued that the action of these compounds might be mediated either directly at 5-HT_{1B} receptors in the SNc or indirectly via an inhibitory GABA-ergic input from SNr to DA neurons. However, this interpretation seems unlikely inasmuch as both drugs were administered systemically, which renders impossible the identification of the precise site of action. In addition, TFMPP is far from being a selective 5-HT_{1B} agonist, in that it was shown to activate also 5-HT_{2C} receptors (Hoyer et al., 1994). On the other hand, we did not find any involvement of this receptor on the regulation of basal firing rate of VTA DA neurons using CGS 12066B (Prisco et al., 1994). It has been shown that 5-HT_{1B} heteroreceptors are located on the axon terminals of GABA-ergic projection neurons from the nucleus accumbens and striatum and not on GABA-ergic interneurons (Waeber and Palacios, 1989; Bruinvels et al., 1994; Sari et al., 1999; Yan and Yan, 2001).

5-HT₂ family

Ugedo et al. (1989) showed that systemic administration of ritanserin, a 5-HT₂ antagonist, was capable of increasing both the firing rate and the bursting activity of DA neurons in the SNc and the VTA. These effects were prevented by 5-HT depletion induced by PCPA (Ugedo et al., 1989). These authors concluded that ‘... *these results suggest that 5-HT exerts an inhibitory control of midbrain DA cell activity mediated by 5-HT₂ receptors* ...’ (Ugedo et al., 1989), obviously

referring to 5-HT₂ as the receptors which were subsequently defined 5-HT_{2A} (Hoyer et al., 1994). However, it is important to point out that the doses of ritanserin (0.5–2.0 mg/kg, i.v.) used in the study of Ugedo et al. (1989) were too high to selectively block 5-HT_{2A} receptors, in that it has been shown that ritanserin is a potent 5-HT antagonist, which also binds with high affinity to 5-HT_{2C} receptors (Boess and Martin, 1994). It is therefore impossible to discriminate the relative contribution of 5-HT_{2A} and 5-HT_{2C} in the disinhibitory effect of ritanserin reported in the study by Ugedo et al. (1989). In a subsequent study, the same research group (Andersson et al., 1995) found that ritanserin (1.0 mg/kg, i.v.) increased the firing rate, the burst firing and the variation coefficient of DA neurons in the VTA but not in the SNc. Moreover, ritanserin pretreatment significantly enhanced the stimulatory effects of low doses of raclopride (10–20 µg/kg, i.v.) on the burst firing of VTA DA neurons (Andersson et al., 1995). These data indicated that unselective blockade of 5-HT₂ receptors by ritanserin can preferentially increase the activity of DA neurons in the VTA, but did not clearly establish which receptor subtype (5-HT_{2A} or 5-HT_{2C} or both?) is involved in this effect. This picture was further complicated by the evidence that ritanserin (0.1–6.4 mg/kg, i.v.) had no consistent effects on the basal firing rate of SNc DA neurons but significantly reversed the inhibition induced by both direct and indirect DA agonists (Shi et al., 1995). However, the effect of ritanserin was apparently mediated by a mechanism independent of 5-HT, but it was due to its ability to selectively block DA autoreceptors (Shi et al., 1995). Although 5-HT_{2A} receptors are localized on a subset of DA cells in the VTA (Nocjar et al., 2002), the use of selective ligands has not revealed a clear role for these receptors in modulating DA-ergic neuronal activity within the nuclei. Blockade of 5-HT_{2A} receptors by the potent and selective 5-HT_{2A} antagonist MDL 100907 [*R*(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (Shi et al., 1995; Minabe et al., 2001) and SR 46349B [but-2-enedioic acid; 4-[(*E*)-3-(2-dimethylaminoethoxyamino)-3-(2-fluorophenyl)prop-2-enylidene]cyclohexa-2,5-dien-1-one]

(Di Giovanni et al., 1999) or their activation with the agonist (\pm)-DOI (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (Di Matteo et al., 2000) had no significant effect on the basal activity of SNc and VTA DA neurons and on their inhibition of direct and indirect DA agonists (Shi et al., 1995). A cell-per-track study showed that MDL 100907 behaves as an atypical antipsychotic (Sorensen et al., 1993). Thus, MDL 100907 (1.0 mg/kg) produced only small increases in the number of active SNc and VTA DA neurons after acute administration (Sorensen et al., 1993) but at 0.1 mg/kg i.p. significantly increased the number of spontaneously active SNc and VTA DA neurons. When administered chronically, MDL 100907 (1.0 mg/kg) selectively reduced the number of spontaneously active VTA neurons (Sorensen et al., 1993), whereas at lower doses (0.03–0.01 mg/kg, i.p.), it was active in reducing the number of the cells in both the DA nuclei.

In spite of these findings, a functional role for 5-HT_{2A} receptors localized on VTA DA neurons has yet to be determined. However, in another study, 5-HT_{2A} antagonists were found to block the inhibitory effect of amphetamine on the basal firing rate of DA neurons, in both the SNc and the VTA (Sorensen et al., 1993) or amphetamine-induced DA release in the nucleus accumbens and striatum (Porrás et al., 2002a). Conversely, the selective blockade of 5-HT_{2A} does not modify the effect of morphine (Porrás et al., 2002a). Thus, this receptor subtype might modulate the activity of both the nigro-striatal and the mesocorticolimbic DA-ergic systems only when specific neuronal circuitry mechanisms are activated. Further investigations into the circuitry of this regulation indicated that 5-HT_{2A} receptors on cortical projections regulate DA cellular activity. 5-HT_{2A} receptors seem to be unable to modulate DA function under resting conditions (Porrás et al., 2002a).

Compelling evidence has been given about the lack of influence on DA cell function by the selective blockade of central 5-HT_{2B} receptors (Di Giovanni et al., 1999; Di Matteo et al., 2000; Gobert et al., 2000).

Fifteen years ago, the issue regarding the role of 5-HT on the control of the electrical activity of DA

neurons in the SNc and the VTA was quite confused and controversial. At that time, a study undertaken in our laboratory shed some light on the subject (Prisco et al., 1994). Systemic administration of mesulergine produced a significant increase in the basal firing rate of VTA DA neurons, whereas ritanserin, used at doses which selectively block 5-HT_{2A} receptors, caused a slight, statistically significant decrease in the basal activity of these neurons (Prisco et al., 1994). These data, although obtained by using partially selective antagonists, represent the first evidence of a differential effect of 5-HT_{2A} and 5-HT_{2C} receptors on DA-containing neurons in the VTA (Prisco et al., 1994). Thus, the data obtained by Prisco et al. (1994) supported the conclusion that 5-HT exerts an inhibitory action on DA neurons in the VTA through the 5-HT_{2C} receptor subtype (which, at that time, was still named 5-HT_{1C}).

Subsequent studies confirmed a selective involvement of 5-HT_{2C} receptors on the basis of the evidence that the inhibitory effect of the mixed 5HT_{2A/2B/2C} receptor agonists mCPP [1-(*meta*-chlorophenyl)piperazine] and MK 212 [6-chloro-2-(1-piperazinyl)piperazine] on the activity of VTA DA-containing neurons and on accumbal DA release was completely prevented by SB 242084 {6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline}, a selective 5-HT_{2C} receptor antagonist (Di Giovanni et al., 2000). Moreover, SB 242084 blocked the inhibitory action of RO 60-0175 [(*S*)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 C₄H₄O₄], a selective 5-HT_{2C} receptor agonist (Di Matteo et al., 2000). Another series of studies have shown that SB 206553 {5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole}, a selective 5-HT_{2C/2B} receptor antagonist, increases the basal firing rate and the bursting activity of VTA DA neurons (Di Giovanni et al., 1999; Gobert et al., 2000). Consistent with these findings, SB 242084 {6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline}, a powerful and selective 5-HT_{2C} receptor antagonist, selectively enhanced the electrical activity of VTA DA neurons, while RO 60-0175 and MK 212, two 5-HT_{2C} receptor agonists, reduced it (Di Matteo et al., 1999). On the other hand, it does not seem

that 5-HT_{2C} receptors exert a relevant role in the control of the nigro-striatal DA-ergic system. Thus, there is evidence that 5-HT_{2C} receptor agonists such as mCPP, MK 212 and RO 60-0175 do not significantly affect the activity of SNc DA neurons (Di Matteo et al., 1999; Di Giovanni et al., 2000). On the other hand, the mixed 5-HT_{2B/2C} antagonist SB 206553 caused only a slight increase in the basal activity of DA neurons in the SNc (Di Giovanni et al., 1999). Consistently, it has been reported that SB 243213 {(5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl] carbamoyl]-6-trifluoromethylindoline hydrochloride)}, a new selective 5-HT_{2C} receptor inverse agonist, at a dose of 3 mg/kg significantly decreased only the number of spontaneously active VTA DA neurons and modified the pattern discharge but did not affect the number of spontaneously active SNc DA cells, whereas the 10 mg/kg dose altered the firing pattern of DA neurons in both the SNc and the VTA (Blackburn et al., 2002).

Therefore, on the basis of the above-mentioned data, it is possible to confirm that the serotonergic system exerts both phasic and tonic control of mesocorticolimbic DA function by acting through 5-HT_{2C} receptors but it seems that it does not tonically affect the nigro-striatal system.

A study carried out in our laboratory has shown that mCPP, systemically and microiontophoretically applied, excites equally all non-DA (presumably GABA-containing) neurons in the VTA by activating 5-HT_{2C} receptors (Di Giovanni et al., 2001). Interestingly, in the SNr, the effect of mCPP and RO 60-0175 was evident only in a subpopulation of SNr neurons (Di Giovanni et al., 2001; Invernizzi et al., 2007): probably the activation of 5-HT_{2C} receptors causes a selective excitation of presumed projection neurons and is ineffective in changing the activity of SNr interneurons synapsing on SNc DA dendrites (Di Giovanni et al., 2001). These findings support our hypothesis of a preferential tonic inhibitory effect of 5-HT through 5-HT_{2C} receptors on the mesocorticolimbic versus the nigro-striatal DA-ergic system.

This highly significant preferential control over the VTA, compared to the nigral DA pathway, by the 5-HT_{2C} receptor subtype, may provide the

rationale for the use of 5-HT_{2C} receptor agonists as antipsychotics, that could improve the mood disorders and cognitive impairments associated with schizophrenia without producing extrapyramidal side-effects (Marquis et al., 2007). In fact, both acute and chronic (21-day) administration of a novel 5-HT_{2C} receptor-selective agonist WAY-163909 (1–10 mg/kg) caused a decrease in the number of spontaneously firing DA neurons in the VTA but not in the SNc. Thus, 5-HT_{2C}-selective receptor agonists might be potential atypical antipsychotics with rapid onset properties (Marquis et al., 2007).

In addition, we have suggested that 5-HT_{2C} receptor antagonists might be useful in the therapy of motor disorders caused by altered DA-ergic function, including those induced by chronic neuroleptic treatment (Di Giovanni et al., 2006a, b; Esposito et al., 2007; Invernizzi et al., 2007). Given that disinhibition of the mesolimbic DA-ergic system may underlie the action of antidepressants, selective 5-HT_{2C} receptor antagonists may also have therapeutic utility in the co-morbid depression in Parkinson's disease (PD) (Di Matteo et al., 2001).

A number of behavioural, neurochemical and electrophysiological studies have shown that 5-HT_{2C} ligands can modulate the pharmacological effects of drug of abuse, including cocaine, nicotine, morphine, amphetamine, ethanol and 9-tetrahydrocannabinol (Porrás et al., 2002a; Pierucci et al., 2004; Bubar and Cunningham, 2006; Ji et al., 2006; Müller and Carey, 2006; chapter 20). Thus, morphine-induced increase in VTA DA neuron firing rate was potentiated by the 5-HT_{2C} receptor antagonist SB 206553 (Porrás et al., 2002a). It was also found that nicotine-induced increase in VTA DA neuronal activity can be prevented by 5-HT_{2C} and that 5-HT_{2C} receptor agonist effects can be induced intracellularly using the protein peptide Tat-3L4F, which prevents 5-HT_{2C} receptor dephosphorylation induced by the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) (Ji et al., 2006; Müller and Carey, 2006). Thus, systemic administration of Tat-3L4F or the 5-HT_{2C} receptor agonist RO 60-0175 suppressed the increased firing rate of VTA DA-ergic neurons

induced by THC, the psychoactive ingredient of marijuana (Ji et al., 2006; Müller and Carey, 2006). Using behavioural tests, it was found that Tat-3L4F or RO 60-0175 blocks conditioned place preference of THC or nicotine, and that RO 60-0175, but not Tat-3L4F, produces anxiogenic effects, thus providing a preclinical basis for treating drug addiction with the Tat-3L4F peptide (Ji et al., 2006; Müller and Carey, 2006; chapter 20).

5-HT₃ subtype

There is little evidence of 5-HT₃ receptor modulation of basal DA firing discharge. Moreover, the few electrophysiological studies carried out at the beginning of the 1990s showed inconsistent results. Several selective ligands for 5-HT₃ receptors are capable of modifying the number of spontaneously active DA neurons, mostly in the VTA. Thus, the selective 5-HT₃ receptor antagonist DAU 6215 [(3- α -tropanyl) 1H-benzimidazolone-3-carboxamide chloride] caused, when given acutely, a significant increase in the number of spontaneously active DA neurons in the VTA but not in the SNc (Prisco et al., 1992). In contrast, repeated consecutive subcutaneous administration of DAU 6215 produced a significant decrease in the number of spontaneously active DA neurons in the VTA but not in the SNc (Prisco et al., 1992). The effect of chronic DAU 6215 on the activity of VTA DA neurons was reversed by apomorphine, suggesting that these neurons were probably in a state of depolarization block (Prisco et al., 1992). Similar results were obtained after chronic administration of the 5-HT₃ receptor antagonist zatosetron (LY 277359, a dihydrobenzofuran) (Rasmussen et al., 1991), but not with MDL 73,147EF, that induced a decreased in number in the SNc as well in the VTA (Sorensen et al., 1989; Palfreyman et al., 1993). However, Ashby et al. (1990) did not reveal any effect of both acute and chronic administration of the 5-HT₃ receptor antagonist BRL 43694 in altering the number of spontaneously active DA cells in either the SNc or the VTA (Ashby et al., 1990). It is possible that this discrepancy is caused by the different selectivity of the antagonists used. Further studies with selective ligands are required

to resolve this question. Since there was evidence that the acute administration of 5-HT₃ receptor antagonists did not alter the basal firing rate of DA neurons in the VTA, but decreased the number of spontaneous cells (Ashby et al., 1990; Minabe et al., 1991; Prisco et al., 1992), it was proposed that these drugs might be useful in the treatment of schizophrenia, behaving as typical or atypical antipsychotics. Nevertheless, this hypothesis was not supported by a trial in the early 1990s that showed the lack of effect of zacopride in a blind study with schizophrenic patients (Newcomer et al., 1992).

5-HT₄ family

Compelling evidence shows that 5-HT₄ receptors exert a state-dependent facilitatory control in vivo on nigro-striatal, but not mesoaccumbal, DA-ergic function. In fact, these receptors appear to modulate the nigro-striatal DA pathway only when DA and 5-HT systems are stimulated. Therefore, systemic administration of the 5-HT₄ antagonist GR125487 {[1-[2-(methylsulphonylamino)ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1-H-indole-3-carboxylate sulphamate} reduced selectively the increases in nigro-striatal cell firing produced by the administration of haloperidol or morphine, but did not alter the response of the VTA neurons (Lucas et al., 2001; Porras et al., 2002b).

5-HT₅₋₆₋₇ subtypes

In recent years, some attention has been paid to the functional importance of 5-HT₅, 5-HT₆ and 5-HT₇ receptors in the pathogenesis of neuropsychiatric and other diseases. In this connection, intensive studies of these receptors with new selective ligands are currently in progress. Notwithstanding, the physiological function of 5-HT₅ receptors is still unclear.

To date, only a single electrophysiological study has examined the effect of the 5-HT₆ antagonist SB 271046 [5-chloro-*N*-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-1-benzothiophene-2-sulphonamide hydrochloride] in the SNc and VTA nuclei (Minabe et al., 2004), although some studies have

evidenced a possible effect in controlling DA release in the mPFC (Lacroix et al., 2004). This in vivo study found complex effects on the DA-ergic neuronal firing pattern and the number of active cells in the VTA. Acute administration of 10 mg/kg of SB 271046 per os (p.o.) leads to a significant decrease in the number of spontaneously active VTA DA neurons but not in the SNc. In addition, this dose was able to decrease the percentage of neurons exhibiting a bursting pattern and the percentage of events as bursts in both areas. The effect in the number of spontaneously active VTA cells disappeared after chronic administration of 10 mg/kg p.o. of SB 271046. This suggests that tolerance develops to the acute effect of the 10 mg/kg of SB 271046, although the drug was capable of significantly decreasing the number of bursts in all spontaneously active VTA DA neurons. In contrast, repeated administration for 21 days of 10 mg/kg of SB 271046 increased the number of cells in the SNc and did not significantly alter the number of spontaneously active VTA DA neurons (Minabe et al., 2004). Thus, based on the in vivo DA cells-per-track model, 5-HT₆ antagonists do not possess antipsychotic efficacy. On the other hand, the interesting ability of increasing the number of spontaneously active SNc DA neurons might be useful in ameliorating the side-effects of typical antipsychotics. However, this has not, so far, been experimentally validated.

Recently, the role of the 5-HT₇ receptor has been investigated (Mnie-Filali et al., 2007). Acute i.p. administration of the selective antagonist SB 269970 [(*R*)-3-(2-(2-(4-methyl-piperidin-1-yl)ethyl)-pyrrolidine-1-sulphonyl)-phenol] did not alter the firing activity (firing and burst rate) of VTA and SNc DA neurons. Interestingly, this antagonist was capable of preventing the inhibition of DA neuronal firing activity induced by amphetamine in the VTA, but not in the SNc. The present results suggest that 5-HT₇ receptors modulate the DA firing activity in the VTA, thus affecting preferentially the mesocorticolimbic pathway. Although definitive explanations of such a discrepancy are not yet provided, a differential modulation of VTA and SNc DA neuronal activity by dorsal raphe 5-HT afferences was reported (Mnie-Filali et al., 2007).

In vitro electrophysiological studies

The peculiarity of DA neurons is that they are also spontaneously active *in vitro* without synaptic input but do not present the typical burst activity seen *in vivo* (Fig. 2). The use of *in vitro* electrophysiological techniques carried on brain slices containing either the SNc or the VTA have offered some advantages compared with *in vivo* methods, including the possibility of studying ionic currents through the membrane of DA and non-DA cells elicited by neurotransmitters, or by other compounds applied locally. However, there are also some limitations linked to the use of *in vitro* methods, such as the cutting of afferent and efferent neuronal inputs to the neurons under study, except for local interneurons contained in the slice of tissue. These methodological differences might explain, at least in part, the discrepant results often reported by *in vitro* and *in vivo* electrophysiological studies regarding the effect of 5-HT on DA neuronal activity in the SNc and the VTA.

One of the first *in vitro* intracellular recordings from SNc DA neurons has shown that iontophoretic application of 5-HT results in an increase in the amplitude of dendritic calcium spikes evoked by direct intracellular depolarization, suggesting the possibility that 5-HT released from serotonergic afferents *in vivo* may modulate nigral DA release through a local action on DA-ergic dendrites (Nedergaard et al., 1988). Thus, this study provided evidence that 5-HT can inhibit DA neuron activity indirectly by stimulating the local release of DA which is known to exert an inhibitory action by acting at SD DA D₂ receptors (Nedergaard et al., 1988). However, no indication about the 5-HT receptor(s) involved in this latter effect was given. Evidence confirmed by *in vitro* extracellular recordings in brain slices containing VTA showed that 5-HT potentiates the inhibitory effect of DA on VTA DA neurons (Brodie and Bunney, 1996). The 5-HT receptor family involved in the modulation of DA D₂ receptor-mediated inhibition seems to be that of 5-HT₂. In fact, Olijslagers et al. (2004) have shown that the 5-HT₂ receptor agonist (\pm) DOI enhanced quinpirole-induced reduction of firing rate of VTA DA

neurons, but not of SNc DA neurons, suggesting that different 5-HT receptor subtypes are involved in modulation of DA D₂-like receptor-mediated inhibition, further supporting the evidence of a different 5-HT₂ control on the two DA-ergic regions. In addition, the selective 5-HT_{2A} receptor antagonist MDL 100907 and the selective 5-HT_{2C} receptor antagonist SB 242084 both abolished the enhancement of quinpirole-induced reduction by either 5-HT or DOI, suggesting the involvement of direct and indirect (possibly via interneurons) modulation pathways in the VTA (Olijslagers et al., 2004). The involvement of 5-HT and specific 5-HT₂ receptors in augmentation of auto-inhibition in the VTA could have important implications for the understanding of the mechanism of atypical antipsychotic drug action (Olijslagers et al., 2006). A possible explanation of this 5-HT₂ effect might be the reduction in amplitude of the hyperpolarization-activated cation current (I_h) in DA neurons. In agreement with this hypothesis, a recent current- and voltage-clamp study demonstrated that 5-HT reduces I_h in VTA cells and this effect is mediated by 5-HT₂ receptors and protein kinase C (Liu et al., 2003). Thus, I_h was reduced by the 5-HT₂ agonist α -Me5-HT, reversed by the 5-HT₂ antagonist ketanserin and blocked by selective protein kinase C inhibitor chelerythrine. The reduction of I_h in DA VTA neurons induced by 5-HT through 5-HT₂ receptors can increase the inhibitory effect of DA on the spontaneous firing rate of DA VTA neurons. Moreover, the reduction of I_h by 5-HT may prolong the postburst after hyperpolarization and reduce excitability of VTA neurons. This more sustained decrease in neuronal excitability might reduce the response to subsequent excitatory stimulation and limit the frequency of bursting in these neurons. Moreover, as burst firing may be induced by excitatory amino acid neurotransmission, this action of 5-HT could have a neuroprotective effect on mesencephalic DA neurons.

Probably, 5-HT₂-mediated D₂ auto-inhibition occurs by activation of G-protein-coupled inwardly rectifying potassium channels (GIRKs), since it has been shown that components of 5-HT₂ signal transduction are able to stabilize the channel in the open state or facilitate $\beta\gamma$ subunit binding

(Olijslagers et al., 2006). Another in vitro study provided further evidence for a 5-HT inhibitory action onto VTA DA neurons; it decreased excitatory postsynaptic currents (EPSCs) and mediated the depressive effect of amphetamine. This is an indirect modulation, through the reduction of the excitatory glutamatergic synaptic transmission onto VTA DA neurons. In this effect, 5-HT₂ receptors do not seem to be involved because ketanserin did not block the effect of amphetamine (Jones and Kauer, 1999). Thus, 5-HT is an important player in the mechanisms underlying drug addiction.

On the other hand, a subsequent set of data shows instead a 5-HT excitatory effect. Intracellular recordings from DA-containing neurons in slices cut from the midbrain of the rat showed that 5-HT reduced the amplitude of GABA_B synaptic potential, thus leading to the conclusion that exogenous 5-HT activates presynaptic 5-HT_{1B} receptors that inhibit the release of GABA onto GABA_B but not GABA_A receptors (Johnson et al., 1992). Intracellular recordings with conventional and with whole-cell patch-clamp electrodes were also made from neurons of the rat VTA and SNc to test the effect of local application of 5-HT and other compounds binding to 5-HT receptor subtypes (Pessia et al., 1994; Cameron et al., 1997). The most common response of DA-containing neurons to 5-HT was depolarization, whereas a small proportion of cells were hyperpolarized (Pessia et al., 1994; Cameron et al., 1997). Application of 5-HT also increased the I_h in some DA cells (Nedergaard et al., 1991; Pessia et al., 1994). Non-DA VTA cells, most probably GABA-ergic interneurons, were depolarized or hyperpolarized by 5-HT (Pessia et al., 1994; Cameron et al., 1997). Depolarizing responses were mimicked by unselective 5-HT₂ receptor agonists but not by unselective 5-HT_{1A} agonists (Pessia et al., 1994). Pessia et al. (1994) concluded that the main direct effect of 5-HT systems on VTA DA neurons might be stimulatory, although this effect would be complemented by indirect changes in DA cell firing resulting from 5-HT exciting or inhibiting GABA-ergic interneurons. The depolarizing effect of 5-HT on VTA DA neurons was confirmed in guinea-pig brain slices, where the

5-HT-releasing agent fenfluramine and the 5-HT_{1D} agonists mimicked the reducing of GABA_B inhibitory postsynaptic potential (IPSP) induced by cocaine treatment (Cameron and Williams, 1994, 1995). In vitro evidence, in addition, shows that administration of CP 93129 or RU 24969, but not 8-OH-DPAT, inhibits high potassium-evoked [³H]GABA release from the VTA slices in a concentration-dependent fashion. This inhibition was antagonized by SB 216641 or cyanopindolol but remained unaffected by the presence of WAY 100635. The results support the hypothesis that 5-HT_{1B} receptors within the VTA, but not 5-HT_{1A}, can function as heteroreceptors to decrease GABA release. Activation of 5-HT_{1B} heteroreceptors localized on 5-HT terminals, or on GABA-ergic interneurons within the VTA, decreases GABA release from either GABA-ergic interneurons, descending GABA projection neurons, or both. This reduction of inhibitory GABA-ergic input into the VTA DA neurons would lead to their disinhibition (Yan and Yan, 2001).

Furthermore, 5-HT potentiates ethanol excitation of DA VTA neurons, and this effect is mediated by 5-HT₂ receptors (Brodie et al., 1995; Trifunovic and Brodie, 1996). Cocaine also potentiates ethanol excitation of these neurons, an effect also mediated by 5-HT₂ receptors (Bunney et al., 2000). In addition, 5-HT can facilitate DA neuron activity shifting the DA neurons towards excitation in response to glutamatergic synaptic input depressing metabotropic glutamate receptor-mediated inhibition postsynaptic current (mGLUR-IPSC) in DA neurons (Paolucci et al., 2003). This effect was not because of a direct modulation of the Ca²⁺-sensitive K⁺ conductances underlying the mGLUR-IPSC, but was associated with a decrease in the intracellular calcium signal triggered by mGLUR stimulation. Similar results were obtained with α -methyl-5-hydroxytryptamine and 5-methoxytryptamine, but not with 5-carboxamidotryptamine or 1-(3-chlorophenyl) piperazine. The powerful inhibition of the mGLUR-IPSC by 5-HT in midbrain DA neurons most probably occurred through stimulation of 5-HT_{2A} and 5-HT₄ receptors, since no significant depression of the mGLUR-IPSC by 5-HT was observed in the presence of the

5-HT₂ antagonist cinanserin, or the 5-HT₄ receptor antagonist RS 23597-190, whereas the 5-HT_{2C} antagonist RS 102221 was ineffective. The depression of the mGLUR-IPSP by 5-HT is associated with a prolongation of the ionotropic glutamate receptor-dependent EPSP. It should also be noted that inhibitory mGLUR-dependent responses are only observed following short high-frequency trains of stimulation that should mimic a burst-like pattern onto the glutamatergic synaptic afferents. Therefore, the serotonergic projection to the SNc should provide a mean of precise and highly selective control of DA release, through a mechanism that is dependent strictly on the firing pattern of afferent glutamatergic fibres. 5-HT could then play an important role in oscillation-dependent processing of information in the basal ganglia, in both physiological and pathological conditions.

Conclusions and future directions

Despite the fact that electrophysiological data regarding the interaction between the serotonergic and the DA-ergic systems in the brain have accumulated over a period of about 35 years of intensive research, the exact effect of 5-HT on the activity of DA neurons is far from being completely understood.

The modulation of DA-ergic activity by the serotonergic system is complex, and complicated by the large number of 5-HT receptor subtypes present in DA nuclei and in their target areas. It can occur both directly and indirectly, through neuronal systems impinging on DA-containing neurons (Fig. 3). Thus, 5-HT acting on receptors on the membrane of SNc and VTA DA neurons can either excite or inhibit them; it can also affect DA neuronal function by modifying the activity of GABA-containing interneurons in the SNc or the VTA. In addition to local circuits, long feed-back loops can be involved in the indirect actions exerted by 5-HT on DA system activity. For example, 5-HT receptors localized either presynaptically on DA terminals or on postsynaptic neurons in the DA projection areas could activate feed-back loops such as the striato-nigral, the accumbens-VTA pathways or the frontal-VTA

pathway, thus indirectly altering the excitability of DA-containing neurons in the SNc or the VTA, which could result in either an increase or a decrease of their basal firing activity and/or firing mode (Fig. 3).

The scenario is also complicated by some experimental caveats. Most of the agonists and antagonists used in the studies reviewed here are selective but are not specific enough to identify receptors involved, especially when used in relatively high concentrations in both in vivo and in vitro physiological experiments. Moreover, most of the in vivo data, obtained by extracellular recordings, are from *putative* DA neurons identified by their firing properties. Nevertheless, these classical identification criteria are deceptive. In fact, the presence of a functionally distinct non-DA-ergic (but not GABA-ergic) population of neurons in the VTA with overlapping characteristics has been demonstrated (Ungless et al., 2004), which is likely to have been included in the analysis of DA neurons in the electrophysiological studies that we reviewed. It is noteworthy that this problem is not idiosyncratic to the in vivo recordings. In in vitro slice preparations, DA neurons are categorized as DA-containing according to the expression of I_h , whereas putative non-DA neurons are not. Importantly, while the absence of an I_h in a DA neuron is a reliable predictor that the cell is not DA-containing (Margolis et al., 2006a, b), the presence of an I_h does not reliably predict TH co-labelling (Margolis et al., 2006a, b). Thus, although the numbers of neurons are small, they provide evidence that a significant number of I_h (+) neurons are not DA-ergic. Therefore, none of the markers that have been assumed to identify midbrain DA-ergic neurons are associated exclusively, or even significantly, with confirmed DA-ergic neurons. Consequently, improved indirect methods of identifying DA neurons need to be developed. Until a more accurate identification scheme is determined for neurons, more care must be taken in attributing properties to putative DA-ergic DA neurons when heterogeneous neural responses are observed in these brain regions.

In addition, the in vivo studies are carried out in anaesthetized rats. Although DA neurons are

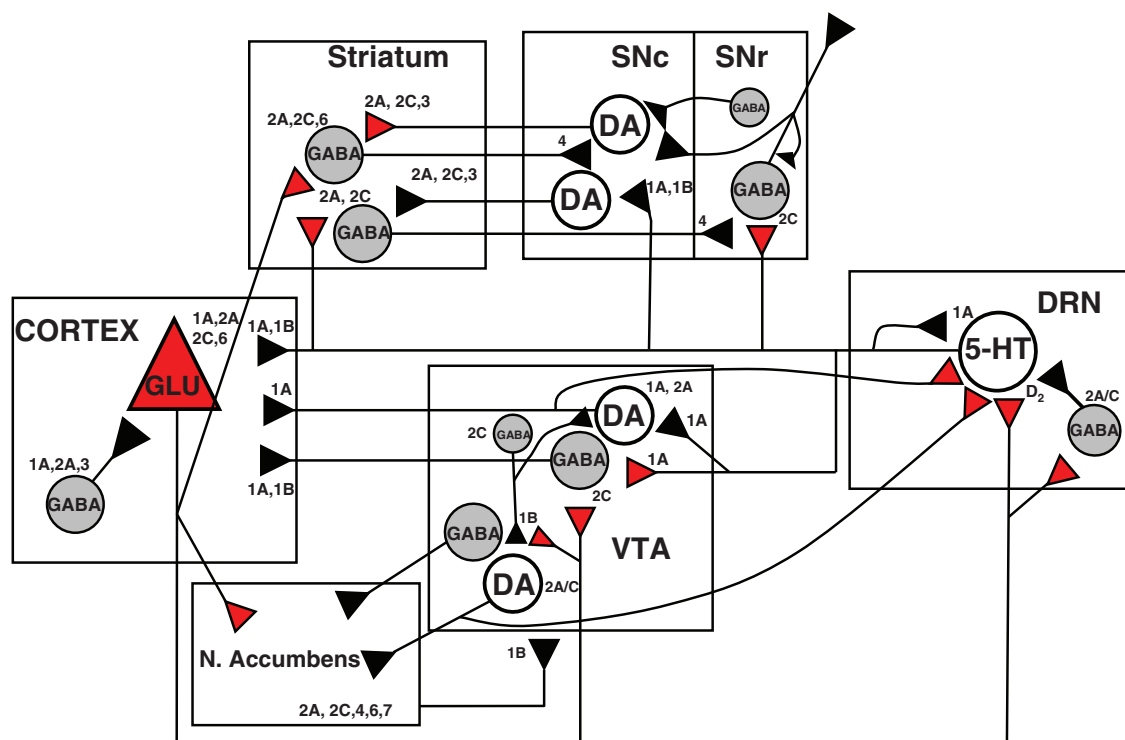


Fig. 3. Schematic representation of the anatomical and functional relationships between the cortex, striatum, nucleus accumbens, VTA, SNc, SNr and the DRN. The possible localization of the different 5-HT receptor subtypes is also shown (see text for more details). Red synaptic buttons are excitatory while those in black are inhibitory. Abbreviations: Glu, glutamate; DA, dopamine; N. Accumbens, nucleus accumbens; GABA, γ -amino-*n*-butyric acid; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine; 1A, 5-HT_{1A}; 1B, 5-HT_{1B}; 2A, 5-HT_{2A}; 2C, 5-HT_{2C}; 3, 5-HT₃; 4, 5-HT₄; 6, 5-HT₆. (See Color Plate 3.3 in the color plate section.)

auto-active, their impulse activity and response to natural neurochemical inputs are strongly affected by general anaesthesia. Some alterations appear to be specific to the general anaesthetic used, while others probably reflect changes in the activity of afferent inputs, brain metabolism and neurotransmitter uptake that are typical to any type of general anaesthesia. Most of the data are obtained using chloral hydrate anaesthesia that is known to exert subtle effects on the basal activity and pharmacological responsiveness of midbrain DA neurons (Kelland et al., 1989). Therefore, taking consideration of the above, when extracellular unit activity from single DA neurons in anaesthetized rats is performed, it is of paramount importance that they are subsequently labelled with the use of the juxtacellular technique, and neurochemically characterized with immunofluorescence for TH.

Moreover, the utilization of microiontophoresis might be encouraged. In fact, despite its methodological constraints, it remains almost the only way of applying relatively few molecules rapidly into the vicinity of central synapses. The technique gets closer to mimicking synaptically released neurotransmitters than any other, and can show direct or indirect effects. As we showed, the conventional analysis of firing and burst rate might be enough to reveal the subtle 5-HT modulator effect on DA neuron (Di Mascio and Esposito, 1997; Di Mascio et al., 1999). 5-HT might also alter spike distribution of DA neurons. Therefore, analysis of the density power spectrum of the signals, the chaos content and the slow oscillation in firing rate (Shi, 2005) should be taken into account when drug effects are evaluated.

Nevertheless, the large body of (often conflicting) literature reviewed here points to the presence of a reciprocal control between the serotonergic systems and the DA-ergic systems both in vitro and in vivo. 5-HT regulates DA neuronal activity in both a tonic and a phasic manner, since its effect is dependent on the activation state of the DA neurons. It is important to emphasize that 5-HT modulation is different in the VTA and the SNc; hence, these two DA populations are far from being a homogenous group. In fact, differential roles appear to exist for some 5-HT receptors. The main effect of 5-HT on DA neurons is an inhibition, a control mediated generally by 5-HT_{2C} receptors, and this applies especially to the VTA. It appears that the inhibitory effect of 5-HT_{2C} receptors is, indirectly, mediated through excitation of GABA-ergic VTA interneurons impinging on DA-containing neurons, although 5-HT_{2C} receptors might be present on VTA DA neurons. Conversely, 5-HT₄ receptors play a more pronounced role in SNc neurons. Moreover, differences in control mechanisms of the GIRK channel by 5-HT receptors in VTA and SNc exist. In fact, 5-HT_{2A}, 5-HT₃ receptors antagonists and 5-HT_{2C} receptor agonists selectively reduce the number of spontaneously active DA neurons in the VTA, but not in the SNc, showing a possible antipsychotic efficacy with reduced risk of extrapyramidal side-effects. Interestingly, 5-HT₆ antagonists increase only the number of SNc DA active neurons. Although they do not possess antipsychotic efficacy, they might be useful in ameliorating the side-effects of typical antipsychotics. On the other hand, the serotonergic system can also excite midbrain DA neurons by a direct depolarizing action or by affecting activity of other areas. More detailed studies on the receptors involved and on their localizations are required to understand the roles of these two opposing effects.

Despite the large body of data available on this subject, there are still a number of points that need to be elucidated. Thus, it would be important to establish the exact brain site(s) involved in the control of DA neuronal activity by the various 5-HT receptor subtypes. For example, it would be important to determine whether or not the effects

of the selective activation of 5-HT subtypes are mediated at the level of the nuclei of origin of the DA-ergic systems (i.e. in the SNc or the VTA), whether or not their effects are direct or indirect (e.g. mediated by GABA-ergic transmission) and whether or not feed-back pathways originating from projection areas of the nigro-striatal and mesocorticolimbic systems are involved in their overall effect on DA neuronal firing rate. Inasmuch as biochemical and neurochemical data have shown that 5-HT_{2A} and 5-HT_{2C} present on nigro-striatal and mesolimbic DA systems have constitutive activity (Clark et al., this volume), it would be intriguing to investigate the presence of constitutive activity also by electrophysiological techniques.

The intensive research in medicinal chemistry will help this field of investigation. In fact, more selective ligands for 5-HT receptors are currently produced. In the future, the use of such selective ligands, especially agonists of 5-HT receptors, would certainly be helpful in determining their functional importance and their involvement in the pathogenesis of diseases, not exclusively of the central nervous system (CNS), but in particular for those disorders such as PD that are still fatal and for which at present there is no cure (Esposito et al., 2007; Di Giovanni, 2008). However, it should be kept in mind that although selective receptor ligands are an important and indispensable research tool, they rarely happen, in practice, to be drugs. Many questions need to be answered before we can truly understand how these 5-HT receptors regulate DA neuronal activity in the brain. The challenge ahead is to build on this foundation and keep up this engaging adventure: the interaction between 5-HT and DA systems is far from being completely revealed.

Abbreviations

Ψ	functional operator equivalent to the density power spectrum of the signals
5,7-DHT	5,7-dihydroxytryptamine
5-HT	5-hydroxytryptamine, serotonin
CNS	central nervous system

DA	dopamine
DRN	dorsal raphe nucleus
EPSC	excitatory postsynaptic current
GABA	γ -amino- <i>n</i> -butyric acid
GAD	glutamic acid decarboxylase
GIRKs	inwardly rectifying potassium channels
GLU	glutamate
I_h	hyperpolarization-activated cation current
IPSP	inhibitory postsynaptic potential
IS-SD	initial segment-somatodendritic
mGLUR	metabotropic glutamate receptor
mPFC	medial prefrontal cortex
MRN	medial raphe nucleus
NRM	nucleus raphe magnus
PCPA	<i>para</i> -chlorophenylalanine
PD	Parkinson's disease
PLA ₂	phospholipase A ₂
PLC	phospholipase C
PTEN	phosphatase and tensin homologue deleted on chromosome 10
SN, SNc, SNr	substantia nigra, substantia nigra pars compacta, substantia nigra pars reticulata
SSRIs	selective serotonin reuptake inhibitors
TH	tyrosine hydroxylase
VTA	ventral tegmental area

References

- Abramowski, D., Rigo, M., Duc, D., Hoyer, D. and Staufenbiel, M. (1995) Localization of the 5-hydroxytryptamine_{2C} receptor protein in human and rat brain using specific antisera. *Neuropharmacology*, 34(12): 1635–1645.
- Aghajanian, G.K. and Bunney, B.S. (1974) DA-ergic and nonDA-ergic neurons of the substantia nigra: differential responses to putative transmitters. In: Boissier J.R., Hippus H. and Pichot P. (Eds.), *Proceedings of the IX Congress of the College of International Neuropsychopharmacology*. Excerpta Medica, Amsterdam, pp. 444–452.
- Aghajanian, G.K. and Bunney, B.S. (1977) Dopamine "autoreceptors": pharmacological characterization by microiontophoretic single cell recording studies. *Naunyn Schmiedeberg Arch. Pharmacol.*, 297(1): 1–7.
- Aman, T.K., Shen, R.Y. and Haj-Dahmane, S. (2007) D2-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J. Pharmacol. Exp. Ther.*, 320(1): 376–385.
- Andersson, J.L., Nomikos, G.G., Marcus, M., Hertel, P., Mathé, J.M. and Svensson, T.H. (1995) Ritanserin potentiates the stimulatory effects of raclopride on neuronal activity and dopamine release selectively in the mesolimbic DA-ergic system. *Naunyn Schmiedeberg Arch. Pharmacol.*, 352(4): 374–385.
- Arborelius, L., Chergui, K., Murase, S., Nomikos, G.G., Höök, B., Chouvet, G., Hacksell, U. and Svensson, T.H. (1993) The 5-HT_{1A} selective ligands, (*R*)-8-OHDPAT and (*S*)-UH-301, differentially affect the activity of midbrain dopamine neurons. *Naunyn Schmiedeberg Arch. Pharmacol.*, 347(4): 353–362.
- Ashby, C.R., Jr., Jiang, L.H. and Wang, R.Y. (1990) Chronic BRL 43694, a selective 5-HT₃ receptor antagonist, fails to alter the number of spontaneously active midbrain dopamine neurons. *Eur. J. Pharmacol.*, 175(3): 347–350.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179(3): 641–659.
- Barnes, J.M., Barnes, N.M., Champaneria, S., Costall, B. and Naylor, R.J. (1990) Characterization and autoradiographic localisation of 5-HT₃ receptor recognition sites identified with [³H]-(*S*)-zacopride in the forebrain of the rat. *Neuropharmacology*, 29(11): 1037–1045.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Beckstead, R.M., Domesick, V.B. and Nauta, W.J. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.*, 175(2): 191–217.
- Berg, K.A., Harvey, J.A., Spampinato, U. and Clarke, W.P. (2005) Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. *Trends Pharmacol. Sci.*, 26(12): 625–630.
- Berretta, N., Freestone, P.S., Guatteo, E., de Castro, D., Geracitano, R., Bernardi, G., Mercuri, N.B. and Lipski, J. (2005) Acute effects of 6-hydroxydopamine on dopaminergic neurons of the rat substantia nigra pars compacta in vitro. *Neurotoxicology*, 26(5): 869–881.
- Blackburn, T.P., Minabe, Y., Middlemiss, D.N., Shirayama, Y., Hashimoto, K. and Ashby, C.R., Jr. (2002) Effect of acute and chronic administration of the selective 5-HT_{2C} receptor antagonist SB-243213 on midbrain dopamine neurons in the rat: an in vivo extracellular single cell study. *Synapse*, 46(3): 129–139.
- Blier, P., de Montigny, C. and Azzaro, A.J. (1986) Effect of repeated amiflamine administration on serotonergic and noradrenergic neurotransmission: electrophysiological studies in the rat CNS. *Naunyn Schmiedeberg Arch. Pharmacol.*, 334(4): 253–260.
- Boess, F.G. and Martin, I.L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology*, 33(3–4): 275–317.
- Boothman, L.J., Mitchell, S.N. and Sharp, T. (2006) Investigation of the SSRI augmentation properties of 5-HT(2) receptor antagonists using in vivo microdialysis. *Neuropharmacology*, 50(6): 726–732.

- Brodie, M.S., Trifunovic, R.D. and Shefner, S.A. (1995) Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains. *J. Pharmacol. Exp. Ther.*, 273(3): 1139–1146.
- Brodie, S.M. and Bunney, E.B. (1996) Serotonin potentiates dopamine inhibition of ventral tegmental area neurons in vitro. *J. Neurophysiol.*, 76(3): 2077–2082.
- Bruinvels, A., Landwehrmeyer, B., Gustafson, E., Durkin, M., Mengod, G., Branchek, T., Hoyer, D. and Palacios, J.M. (1994) Localisation of 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} receptor messenger RNA in the rodent and primate brain. *Neuropharmacology*, 33(3–4): 367–386.
- Bubar, M.J. and Cunningham, K.A. (2006) Serotonin 5-HT_{2A} and 5-HT_{2C} receptors as potential targets for modulation of psychostimulants use and dependence. *Curr. Top. Med. Chem.*, 6(18): 1971–1985.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Bunney, B.S., Aghajanian, G.K. and Roth, R.H. (1973a) Comparison of effects of L-Dopa, amphetamine and apomorphine on firing rate of rat DA-ergic neurones. *Nat. New Biol.*, 245(143): 123–125.
- Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G.K. (1973b) DA-ergic neurons: effects of antipsychotic drugs and amphetamine on single cell activity. *J. Pharmacol. Exp. Ther.*, 185: 560–571.
- Bunney, E.B., Appel, S.B. and Brodie, M.S. (2000) Cocaine potentiates ethanol-induced excitation of DA-ergic reward neurons in the ventral tegmental area. *J. Pharmacol. Exp. Ther.*, 293(2): 383–389.
- Cameron, D. and Williams, J. (1995) Opposing roles for dopamine and serotonin at presynaptic receptors in the ventral tegmental area. *Clin. Exp. Pharmacol. Physiol.*, 22: 841–845.
- Cameron, D.L., Wessendorf, M.W. and Williams, J.T. (1997) A subset of ventral tegmental area neurons is inhibited by dopamine, 5-hydroxytryptamine and opioids. *Neuroscience*, 77: 155–166.
- Cameron, D.L. and Williams, J.T. (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. *J. Neurosci.*, 14: 6763–6767.
- Chiodo, L.A. (1988) Dopamine-containing neurons in the mammalian central nervous system: electrophysiology and pharmacology. *Neurosci. Biobehav. Rev.*, 12(1): 49–91.
- Chiodo, L.A. and Bunney, B.S. (1985) Possible mechanisms by which repeated clozapine administration differentially affects the activity of two subpopulations of midbrain dopamine neurons. *J. Neurosci.*, 5(9): 2539–2544.
- Chu, Y.X., Liu, J., Feng, J., Wang, Y., Zhang, Q.J. and Li, Q. (2004) Changes of discharge rate and pattern of 5-hydroxytryptamine neurons of dorsal raphe nucleus in a rat model of Parkinson's disease. *Sheng Li Xue Bao*, 56(5): 597–602.
- Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P. and Fone, K.C.F. (2000) Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. *Neuropharmacology*, 39: 123–132.
- Cornea-Hebert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin_{2A} receptor in the central nervous system. *J. Comp. Neurol.*, 409: 187–209.
- Corvaja, N., Doucet, G. and Bolam, J.P. (1993) Ultrastructure and synaptic targets of the raphe-nigral projection in the rat. *Neuroscience*, 103: 417–427.
- Cremers, T.I., Giorgetti, M., Bosker, F.J., Hogg, S., Arnt, J., Mørk, A., Honig, G., Bøgesø, K.P., Westerink, B.H., den Boer, H., Wikström, H.V. and Tecott, L.H. (2004) Inactivation of 5-HT(2C) receptors potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology*, 29(10): 1782–1789.
- Cremers, T.I., Rea, K., Bosker, F.J., Wikström, H.V., Hogg, S., Mørk, A. and Westerink, B.H. (2007) Augmentation of SSRI effects on serotonin by 5-HT_{2C} antagonists: mechanistic studies. *Neuropsychopharmacology*, 32(7): 1550–1557.
- Decavel, C., Lescaudron, L., Mons, N. and Calas, A. (1987) First visualization of dopaminergic neurons with a monoclonal antibody to dopamine: a light and electron microscopic study. *J. Histochem. Cytochem.*, 35(11): 1245–1251.
- Diaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of DA-ergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25(47): 10831–10843.
- Di Giovanni, G. (2008) Will it ever become possible to prevent dopaminergic neuronal degeneration? *CNS Neurol. Disord. Drug Targets*, 7(1): 28–44.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin_{2C/2B} receptors enhances mesolimbic and mesostriatal DA-ergic function: a combined *in vivo* electrophysiological and microdialysis study. *Neuroscience*, 91: 587–597.
- Di Giovanni, G., Di Matteo, V., Di Mascio, M. and Esposito, E. (2000) Preferential modulation of mesolimbic versus nigrostriatal DA-ergic function by serotonin_{2C/2B} receptor agonists: a combined *in vivo* electrophysiological and microdialysis study. *Synapse*, 35: 53–61.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) *m*-Chlorophenylpiperazine excites non-DA-ergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience*, 103: 111–116.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006a) Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT_{2C} receptor be a new target for therapeutic strategies? *Curr. Med. Chem.*, 13(25): 3069–3081.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006b) Central serotonin_{2C} receptor: from physiology to pathology. *Curr. Top. Med. Chem.*, 6(18): 1909–1925.

- Di Mascio, M., Di Giovanni, G., Di Matteo, V. and Esposito, E. (1999) Decreased chaos of midbrain DA-ergic neurons after serotonin denervation. *Neuroscience*, 91(2): 587–597.
- Di Mascio, M., Di Giovanni, G., Di Matteo, V., Prisco, S. and Esposito, E. (1998) Selective serotonin reuptake inhibitors reduce the spontaneous activity of DA-ergic neurons in the ventral tegmental area. *Brain Res. Bull.*, 46(6): 544–547.
- Di Mascio, M. and Esposito, E. (1997) The degree of inhibition of dopaminergic neurons in the ventral tegmental area induced by selective serotonin reuptake inhibitors is a function of the density-power-spectrum of the interspike interval. *Neuroscience*, 79: 957–961.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22(5): 229–232.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases DA-ergic transmission in the mesolimbic system. *Neuropharmacology*, 38: 1195–1205.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic DA-ergic function through serotonin_{2C} receptors. *Brain Res.*, 865(1): 85–90.
- Doherty, M.D. and Pickel, V.M. (2000) Ultrastructural localization of the serotonin 2A receptor in DA-ergic neurons in the ventral tegmental area. *Brain Res.*, 864: 176–185.
- Doherty, M.D. and Pickel, V.M. (2001) Targeting of serotonin 1A receptors to DA-ergic neurons within the parabrachial subdivision of the ventral tegmental area in the rat brain. *J. Comp. Neurol.*, 433: 390–400.
- Dray, A., Davies, J., Oakley, N.R., Tongroach, P. and Vellucci, S. (1978) The dorsal and medial raphe projections to the substantia nigra in the rat: electrophysiological, biochemical and behavioural observations. *Brain Res.*, 151: 431–442.
- Dray, A., Gonye, N., Oakley, N.R. and Tanner, T. (1976) Evidence for the existence of a raphe projection to the substantia nigra in the rat. *Brain Res.*, 113: 45–57.
- Duxon, M.S., Flanagan, T.P., Revley, A.C., Baxter, G.S., Blackburn, T.P. and Fone, C.F. (1997) Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. *Neuroscience*, 76(2): 323–329.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.-F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384: 233–247.
- Esposito, E., Di Matteo, V., Pierucci, M., Benigno, A. and Di Giovanni, G. (2007) Role of central 5-HT_{2C} receptor in the control of basal ganglia functions. In: Di Giovanni G. (Ed.), *The Basal Ganglia Pathophysiology: Recent Advances*. Transworld Research Network, Trivandrum, pp. 97–127.
- Fahn, S., Libsch, L.R. and Cutler, R.W. (1971) Monoamines in the human neostriatum; topographic distribution in normals and in Parkinson's disease and their role in akinesia, rigidity, chorea and tremor. *J. Neurol. Sci.*, 14: 427–455.
- Felder, C.C., Kanterman, R.Y., Ma, A.L. and Axelrod, J. (1990) Serotonin stimulates phospholipase A₂ and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc Natl. Acad. Sci. U.S.A.*, 87(6): 2187–2191.
- Ferre, S. and Artigas, F. (1993) Dopamine D₂ receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. *J. Neurochem.*, 61: 772–775.
- Fibiger, H.C. and Miller, J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience*, 2: 975–987.
- Fuxe, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. *Acta Physiol. Scand.*, 247(Suppl.): 39–85.
- Gervais, J. and Rouillard, C. (2000) Dorsal raphe stimulation differentially modulates DA-ergic neurons in the ventral tegmental area and substantia nigra. *Synapse*, 35: 281–291.
- Gobert, A., Rivet, J.-M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.-P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical DA-ergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36: 205–221.
- Grace, A.A. and Bunney, B.S. (1980) Nigral dopamine neurons: intracellular recordings and identification with L-Dopa injection and histofluorescence. *Science*, 210: 654–656.
- Grace, A.A. and Bunney, B.S. (1983a) Intracellular and extracellular electrophysiology of nigral DA-ergic neurons — 1. Identification and characterization. *Neuroscience*, 10(2): 301–315.
- Grace, A.A. and Bunney, B.S. (1983b) Intracellular and extracellular electrophysiology of nigral DA-ergic neurons — 2. Action potential generating mechanisms and morphological correlates. *Neuroscience*, 10(2): 317–331.
- Grace, A.A. and Bunney, B.S. (1984) The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.*, 4: 2877–2890.
- Grace, A.A. and Bunney, B.S. (1986) Injection of depolarization block in midbrain dopamine neurons by repeated administration of haloperidol: analysis using in vivo intracellular recording. *J. Pharmacol. Exp. Ther.*, 238(3): 1092–1100.
- Guiard, B.P., El Mansari, M., Merali, Z. and Blier, P. (2008) Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *Int. J. Neuropsychopharmacol.*, 1–15.
- Haj-Dahmane, S. (2001) D₂-like dopamine receptor activation excites rat dorsal raphe 5-HT neurons in vitro. *Eur. J. Neurosci.*, 14: 125–134.

- Hervé, D., Pickel, V.M., Joh, T.H. and Beaudet, A. (1987) Serotonin axon terminals in the ventral tegmental area and synaptic input to DA-ergic neurons. *Brain Res.*, 435: 71–83.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humprey, P.P. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, 46: 157–203.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M. (1986) Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT₂ recognition sites. *Brain Res.*, 376: 97–107.
- Ikemoto, K., Nishimura, A., Okado, N., Mikuni, M., Nishi, K. and Nagatsu, I. (2000) Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. *Brain Res.*, 853: 377–380.
- Invernizzi, R.W., Pierucci, M., Calcagno, E., Di Giovanni, G., Di Matteo, V., Benigno, A. and Esposito, E. (2007) Selective activation of 5-HT_{2C} receptors stimulated GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience*, 144: 1523–1535.
- Ji, S.-P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R. and Zhang, X. (2006) Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat. Med.*, 12: 324–329.
- Johnson, S.W., Mercuri, N.B. and North, R.A. (1992) 5-Hydroxytryptamine_{1B} receptors block the GABA_B synaptic potential in rat dopamine neurons. *J. Neurosci.*, 12(5): 2000–2006.
- Jones, S. and Kauer, J.A. (1999) Amphetamine depresses excitatory synaptic transmission via serotonin receptors in the ventral tegmental area. *J. Neurosci.*, 15: 9780–9787.
- Kalén, P., Skagerberg, G. and Lindvall, O. (1988) Projections from the ventral tegmental area and mesencephalic raphe to the dorsal raphe nucleus in the rat. Evidence for a minor DA-ergic component. *Exp. Brain Res.*, 73(1): 69–77.
- Kelland, M.D., Freeman, A.S. and Chiodo, L.A. (1989) Chloral hydrate anaesthesia alters the responsiveness of identified midbrain dopamine neurons to dopamine agonist administration. *Synapse*, 3(1): 30–37.
- Kelland, M.D., Freeman, A.S. and Chiodo, L.A. (1990) Serotonergic afferent regulation of the basic physiology and pharmacological responsiveness of nigrostriatal dopamine neurons. *J. Pharmacol. Exp. Ther.*, 253(2): 803–811.
- Kelland, M.D., Freeman, A.S., Rubin, J. and Chiodo, L.A. (1993) Ascending afferent regulation of rat midbrain dopamine neurons. *Brain Res. Bull.*, 31: 539–546.
- Kilpatrick, G., Russell, M.H. and Gale, J.D. (1996) 5-HT₃ and 5-HT₄ receptors in terminal regions of the mesolimbic system. *Behav. Brain Res.*, 73: 11–13.
- Kitahama, K., Nagatsu, I., Geffard, M. and Maeda, T. (2000) Distribution of dopamine-immunoreactive fibers in the rat brainstem. *J. Chem. Neuroanat.*, 18(1–2): 1–9.
- Lacroix, L.P., Dawson, L.A., Hagan, J.J. and Heidbreder, C.A. (2004) 5-HT₆ receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. *Synapse*, 51: 158–164.
- Lavoie, B. and Parent, A. (1990) Immunohistochemical study of the serotonergic innervation of the basal ganglia in the squirrel monkey. *J. Comp. Neurol.*, 299: 1–16.
- Lejeune, F. and Millan, M.J. (1998) Induction of burst firing in ventral tegmental area DA-ergic neurons by activation of serotonin (5-HT)_{1A} receptors: WAY 100,635-reversible actions of the highly selective ligands, flesinoxan and S 15535. *Synapse*, 30(2): 172–180.
- Liu, S., Bubar, M.J., Lanfranco, M.F., Hillman, G.R. and Cunningham, K.A. (2007) Serotonin_{2C} receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*, 146(4): 1677–1688.
- Liu, Z., Bunney, E.B., Appel, S.B. and Brodie, M.S. (2003) Serotonin reduces the hyperpolarization-activated current (I_h) in ventral tegmental area dopamine neurons: involvement of 5-HT₂ receptors and protein kinase C. *J. Neurophysiol.*, 90(5): 3201–3212.
- Lucas, G., Di Matteo, V., De Deurwaerdere, P., Porras, G., Martin-Ruiz, R., Artigas, F., Esposito, E. and Spampinato, U. (2001) Neurochemical and electrophysiological evidence that 5-HT₄ receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal, dopaminergic function. *Eur. J. Neurosci.*, 13: 889–898.
- Mackay, A.V.P., Yates, C.M., Wright, A., Hamilton, P. and Davies, P. (1978) Regional distribution of monoamines and their metabolites in the human brain. *J. Neurochem.*, 30: 841–846.
- Maj, J. and Moryl, E. (1992) Effects of sertraline and citalopram given repeatedly on the responsiveness of 5-HT receptor subpopulations. *J. Neural Transm. Gen. Sec.*, 88: 143–156.
- Mansour, A., Meador-Woodruff, J.H., Bunzow, J.R., Civelli, O., Akil, H. and Watson, S.J. (1990) Localization of D₂ receptor mRNA and D₁ and D₂ receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. *J. Neurosci.*, 10: 2587–2600.
- Marazziti, D., Rossi, A., Giannaccini, G., Zavaglia, K.M., Dell'Osso, L., Lucacchini, A. and Cassano, G.B. (1999) Distribution and characterization of [³H]mesulergine binding in the human brain postmortem. *Eur. Neuropsychopharmacol.*, 10: 21–26.
- Margolis, E.B., Lock, H., Chefer, V.I., Shippenberg, T.S., Hjelmstad, G.O. and Fields, H.L. (2006a) Kappa opioids selectively control dopaminergic neurons projecting to the prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 103: 2938–2942.
- Margolis, E.B., Lock, H., Hjelmstad, G.O. and Fields, H.L. (2006b) The ventral tegmental area revisited: is there an

- electrophysiological marker for dopaminergic neurons? *J. Physiol.*, 577(Pt. 3): 907–924.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Jr., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]: a novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.
- Martin, G.R. and Humphrey, P.P.A. (1994) Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology*, 33: 261–273.
- Martin-Ruiz, R., Ugedo, L., Honrubia, M.A., Mengod, G. and Artigas, F. (2001) Control of serotonergic neurons in the rat brain by DA-ergic receptors outside the dorsal raphe nucleus. *J. Neurochem.*, 77: 762–775.
- Mengod, G., Nguyen, H., Le, H., Waeber, C., Lübbert, H. and Palacios, J.M. (1990a) The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. *Neuroscience*, 35(3): 577–591.
- Mengod, G., Pompeiono, M., Martinez-Mir, I. and Palacios, J.M. (1990b) Localization of the mRNA for the 5-HT₂ receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res.*, 524: 139–143.
- Minabe, Y., Emori, K. and Ashby, C.R., Jr. (1996) The depletion of brain serotonin levels by *para*-chlorophenylalanine administration significantly alters the activity of midbrain dopamine cells in rats: an extracellular single cell recording study. *Synapse*, 22(1): 46–53.
- Minabe, Y., Hashimoto, K., Watanabe, K.I. and Ashby, C.R., Jr. (2001) Acute and repeated administration of the selective 5-HT(2A) receptor antagonist M100907 significantly alters the activity of midbrain dopamine neurons: an in vivo electrophysiological study. *Synapse*, 40(2): 102–112.
- Minabe, Y., Shirayama, Y., Hashimoto, K., Routledge, C., Hagan, J.J. and Ashby, C.R., Jr. (2004) Effect of the acute and chronic administration of the selective 5-HT₆ receptor antagonist SB-271046 on the activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. *Synapse*, 52: 20–28.
- Minabe, Y.M., Ashby, C.R., Jr., Schwartz, J.E. and Wang, R.Y. (1991) The 5-HT₃ receptor antagonists LY 277359 and granisetron potentiate the suppressant action of apomorphine on the basal firing rate of ventral tegmental dopamine cells. *Eur. J. Pharmacol.*, 209: 143–150.
- Mnie-Filali, O., Dahan, L., Zimmer, L. and Haddjeri, N. (2007) Effects of the serotonin 5-HT(7) receptor antagonist SB-269970 on the inhibition of dopamine neuronal firing induced by amphetamine. *Eur. J. Pharmacol.*, 570(1–3): 72–76.
- Molineaux, S.M., Jessel, T.M., Axel, R. and Julius, D. (1989) 5-HT_{1c} receptor is a prominent receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.*, 86: 6793–6797.
- Mori, S., Matsuura, T., Takino, T. and Sano, Y. (1987) Light and electron microscopic immunohistochemical studies of serotonin nerve fibers in the substantia nigra of the rat, cat and monkey. *Anat. Embryol.*, 176: 13–18.
- Morilak, D.A., Garlow, S.J. and Ciaranello, R.D. (1993) Immunocytochemical localization and description of neurons expressing serotonin₂ receptors in the rat brain. *Neuroscience*, 54(3): 701–717.
- Moukhlès, H., Bosler, O., Bolam, J.P., Vallée, A., Umbriaco, D., Geffards, M. and Doucet, G. (1997) Quantitative and morphometric data indicate precise cellular interactions between serotonin terminals and postsynaptic targets in the substantia nigra. *Neuroscience*, 76(4): 1159–1171.
- Müller, C.P. and Carey, R.J. (2006) Intracellular 5-HT_{2C}-receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol. Sci.*, 27(9): 455–458.
- Nedergaard, S., Bolam, J.P. and Greenfield, S.A. (1988) Facilitation of a dendritic conductance by 5-hydroxytryptamine in the substantia nigra. *Nature*, 333: 174–177.
- Nedergaard, S., Flatman, J.A. and Engberg, I. (1991) Excitation of substantia nigra pars compacta neurons by 5-hydroxytryptamine in vitro. *Neuroreport*, 2: 329–332.
- Newcomer, J.W., Faustman, W.O., Zipursky, R.B. and Csernansky, J.G. (1992) Zucopride in schizophrenia: a single-blind serotonin type 3 antagonist trial. *Arch. Gen. Psychiatry*, 49: 751–752.
- Nocjar, C., Roth, B.L. and Pehek, E.A. (2002) Localization of 5-HT_{2A} receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, 111(1): 163–176.
- Olijslagers, J.E., Werkman, T.R., McCreary, A.C., Kruse, C.G. and Wadman, W.J. (2006) Modulation of midbrain dopamine neurotransmission by serotonin, a versatile interaction between neurotransmitters and significance for antipsychotic drug action. *Curr. Neuropharmacol.*, 4: 59–68.
- Olijslagers, J.E., Werkman, T.R., McCreary, A.C., Siarey, R., Kruse, C.G. and Wadman, W.J. (2004) 5-HT₂ receptors differentially modulate dopamine-mediated auto-inhibition in A9 and A10 midbrain areas of the rat. *Neuropharmacology*, 46(4): 504–510.
- Palfreyman, M.G., Schmidt, C.J., Sorensen, S.M., Dudley, M.W., Khene, J.H., Moser, P., Gittos, M.W. and Carr, A.A. (1993) Electrophysiological, biochemical, and behavioral evidence for 5-HT₂ and 5-H₃ mediated control of DA-ergic function. *Psychopharmacology*, 112: S60–S67.
- Palkovits, M., Brownstein, M. and Saavedra, J.M. (1974) Serotonin content of the brain stem nuclei in the rat. *Brain Res.*, 80: 237–249.
- Paolucci, E., Berretta, N., Tozzi, A., Bernardi, G. and Mercuri, N.B. (2003) Depression of mGluR-mediated IPSCs by 5-HT in dopamine neurons of the rat substantia nigra pars compacta. *Eur. J. Neurosci.*, 18(10): 2743–2750.
- Pasqualetti, M., Ori, M., Castagna, M., Marazziti, D., Cassano, G.B. and Nardi, I. (1999) Distribution and cellular

- localization of the serotonin type 2C receptor messenger RNA in the human brain. *Neuroscience*, 92(2): 601–611.
- Pazos, A., Cortés, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.*, 346: 231–249.
- Pazos, A. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, 346: 205–230.
- Pazos, A., Probst, A. and Palacios, J.M. (1987) Serotonin receptors in the human brain — III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience*, (1): 97–122.
- Pessia, M., Jiang, Z.G., North, R.A. and Johnson, S.W. (1994) Actions of 5-hydroxytryptamine on ventral tegmental area neurons of the rat *in vitro*. *Brain Res.*, 654: 324–330.
- Peyron, C., Luppi, P.H., Kitahama, K., Fort, P., Hermann, D.M. and Jouvet, M. (1995) Origin of the DA-ergic innervation of the rat dorsal raphe nucleus. *Neuroreport*, 6(18): 2527–2531.
- Pierucci, M., Di Matteo, V. and Esposito, E. (2004) Stimulation of serotonin_{2C} receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.*, 309: 109–118.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Brain Res. Mol. Brain Res.*, 23: 163–178.
- Porras, G., Di Matteo, V., De Deurwaerdère, P., Esposito, E. and Spampinato, U. (2002a) Central serotonin₄ receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: *in vivo* studies with morphine, amphetamine and cocaine. *Neuropharmacology*, 43: 1099–1109.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdère, P., Caccia, C., Esposito, E. and Spampinato, U. (2002b) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced *in vivo* by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 311–324.
- Prisco, S. and Esposito, E. (1995) Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of DA-ergic neurons in the ventral tegmental area. *Br. J. Pharmacol.*, 116: 1923–1931.
- Prisco, S., Pagannone, S. and Esposito, E. (1994) Serotonin-dopamine interaction in the ventral tegmental area: an electrophysiological study *in vivo*. *J. Pharmacol. Exp. Ther.*, 271(1): 83–90.
- Prisco, S., Pessia, M., Ceci, A., Borsini, F. and Esposito, E. (1992) Chronic treatment with DAU 6215, a new 5-HT₃ receptor antagonist, causes a selective decrease in the number of spontaneously active DA-ergic neurons in the rat ventral tegmental area. *Eur. J. Pharmacol.*, 214: 13–19.
- Rasmussen, K., Stockton, M.E. and Czachura, J.F. (1991) The 5-HT₃ receptor antagonist zatosetron decreases the number of spontaneously active A10 dopamine neurons. *Eur. J. Pharmacol.*, 205: 113–116.
- Saavedra, J.M. (1977) Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. *Fed. Proc.*, 36: 2134–2141.
- Sakai, K., Salvat, D., Touret, M. and Jouvet, M. (1977) Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. *Brain Res.*, 137(1): 11–35.
- Sari, Y., Miquel, M., Brisorgueil, M., Ruiz, G., Doucet, E., Hamon, M. and Vergé, D. (1999) Cellular and subcellular localization of 5-hydroxytryptamine_{1B} receptors in the rat central nervous system: immunocytochemical autoradiographic and lesion studies. *Neuroscience*, 88: 899–915.
- Sekine, Y., Suzuki, K., Ramachandran, P.V., Blackburn, T.P. and Ashby, C.R., Jr. (2007) Acute and repeated administration of fluoxetine, citalopram, and paroxetine significantly alters the activity of midbrain dopamine neurons in rats: an *in vivo* electrophysiological study. *Synapse*, 61(2): 72–77.
- Shannak, K.S. and Ornykiewicz, O. (1980) Brain monoamines in the rhesus monkey during long-term neuroleptic administration. *Adv. Biochem. Psychopharmacol.*, 24: 315–323.
- Shi, W.-X. (2005) Slow oscillatory firing: a major firing pattern of dopamine neurons in the ventral tegmental area. *J. Neurophysiol.*, 94(5): 3516–3522.
- Shi, W.-X., Nathaniel, P. and Bunney, B.S. (1995) Ritanserin, a 5-HT_{2A/2C} antagonist, reverses direct dopamine agonist-induced inhibition of midbrain dopamine neurons. *J. Pharmacol. Exp. Ther.*, 274: 735–740.
- Sinton, C.M. and Fallon, S.L. (1988) Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT₁ receptor. *Eur. J. Pharmacol.*, 157: 173–181.
- Sorensen, S.M., Humphreys, T.M. and Palfreyman, M.G. (1989) Effect of acute and chronic MDL 73,147EF, a 5-HT₃ receptor antagonist, on A9 and A10 dopamine neurons. *Eur. J. Pharmacol.*, 163: 115–118.
- Sorensen, S.M., Kehne, J.H., Fayadel, G.M., Humphreys, T.M., Ketteler, H.J., Sullivan, C.K., Taylor, V.L. and Schmidt, C.J. (1993) Characterization of the 5-HT₂ receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioural, electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.*, 266(2): 684–691.
- Steinbusch, H.W.M. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4): 557–618.
- Suzuki, M., Hurd, Y.L., Sokoloff, P., Schwartz, J.C. and Sedvall, G. (1998) D₃ dopamine receptor mRNA is widely expressed in the human brain. *Brain Res.*, 779: 58–74.
- Svensson, T.H., Bunney, B.S. and Aghajanian, G.K. (1975) Inhibition of both noradrenergic and serotonergic neurons in the brain by the alpha-adrenergic agonist clonidine. *Brain Res.*, 92: 291–306.
- Tepper, J.M., Sawyer, S.F. and Groves, P.M. (1987) Electrophysiologically identified nigral DA-ergic neurons intracellularly labeled with HRP: light-microscopic analysis. *J. Neurosci.*, 7(9): 2794–2806.

- Trent, F. and Tepper, J.M. (1991) Dorsal raphe stimulation modifies striata-evoked antidromic invasion of nigral dopaminergic neurons in vivo. *Exp. Brain Res.*, 84: 620–630.
- Trifunovic, R.D. and Brodie, M.S. (1996) The effects of clomipramine on the excitatory action of ethanol on DA-ergic neurons of the ventral tegmental area in vitro. *J. Pharmacol. Exp. Ther.*, 276: 34–40.
- Ugedo, L., Grenhoff, J. and Svensson, T.H. (1989) Ritanserin, a 5-HT₂ receptor antagonist, activates midbrain dopamine neurons by blocking serotonin inhibition. *Psychopharmacology*, 98: 45–50.
- Ungless, M.A., Magill, P.J. and Bolam, J.P. (2004) Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science*, 303(5666): 2040–2042.
- Van Bockstaele, E.J., Cestari, D.M. and Pickel, V.M. (1994) Synaptic structure and connectivity of serotonin area: potential sites for modulation of mesolimbic dopamine neurons. *Brain Res.*, 647: 307–322.
- Van Bockstaele, E.J. and Pickel, V.M. (1994) Ultrastructure of serotonin-immunoreactive terminals in the core and shell of the rat nucleus accumbens: cellular substrates for interactions with catecholamine afferents. *J. Comp. Neurol.*, 334: 603–617.
- Vertes, R.P. and Linley, S.B. (2007) Comparison of the dorsal and median raphe nuclei, with some functional considerations. *Int. Congr. Ser.*, 1304: 98–120.
- Waeber, C. and Palacios, J.M. (1989) Serotonin-1 receptor binding sites in the human basal ganglia are decreased in Huntington's chorea but not in Parkinson's disease: a quantitative in vitro autoradiography study. *Neuroscience*, 32: 337–347.
- Wang, R.Y. (1981) DA-ergic neurons in the rat ventral tegmental area. Identification and characterization. *Brain Res. Rev.*, 3: 123–140.
- Ward, R.P. and Dorsa, D.M. (1996) Colocalization of serotonin receptor subtypes 5-HT_{2A}, 5-HT_{2C}, and 5-HT₆ with neuropeptides in the rat striatum. *J. Comp. Neurol.*, 370: 405–414.
- Wirtshafter, D., Stratford, T.R. and Asin, K.E. (1987) Evidence that serotonergic projections to the substantia nigra arise in the dorsal, but not the median, raphe nucleus. *Neurosci. Lett.*, 77: 261–266.
- Wright, D.E., Serogy, K.B., Lundgren, K.H., Davis, B.N. and Jennes, L. (1995) Comparative localization of serotonin_{1A}, _{1C}, and ₂ receptor subtype mRNAs in the rat brain. *J. Comp. Neurol.*, 351: 357–373.
- Yan, Q.-S. and Yan, S.-E. (2001) Serotonin-1B receptor-mediated inhibition of [³H]GABA release from rat ventral tegmental area slices. *J. Neurochem.*, 79: 914–922.
- Zhang, Q.J., Gao, R., Liu, J., Liu, Y.P. and Wang, S. (2007) Changes in the firing activity of serotonergic neurons in the dorsal raphe nucleus in a rat model of Parkinson's disease. *Sheng Li Xue Bao*, 59(2): 183–189.

CHAPTER 4

Functional genetic polymorphisms in serotonin and dopamine gene systems and their significance in behavioural disorders

Ursula M. D'Souza* and Ian W. Craig

*MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre, Institute of Psychiatry, King's College London,
De Crespigny Park, Denmark Hill, London SE5 8AF, UK*

Abstract: Many genes in the monoamine neurotransmitter pathways possess functional variants which have been associated with human behavioural disorders and traits, making them of important clinical relevance. In this chapter, we summarize the most recent literature concerning functional studies on these variants and their possible behavioural consequences. Such studies have adopted a variety of strategies. Key investigations have determined effects on gene expression at the level of transcription in mammalian cell cultures, human lymphoblasts and/or human post-mortem brain tissue employing a range of strategies including allele-specific expression. This has enabled the comparison of in vitro and in vivo data, and furthermore provides an improved perceptive of their respective advantages. Pharmacological studies have focused on the effects of gene variation at the protein level in terms of binding to ligands and drugs. Additionally, molecular biological approaches have identified transcription factors (DNA-binding proteins) that interact with the motifs within the polymorphisms themselves. Various neuroimaging studies have further determined the relationship of genotype with protein availability in the brain, thereby contributing further to an understanding of the in vivo functional significance of gene variants. Finally, there is growing evidence from both human and animal studies on the interaction of functional polymorphisms with the environment in determining behavioural outcomes. Taken together, these findings have contributed to a greater understanding of the plausible molecular mechanisms underpinning the functional significance of polymorphisms in monoamine neurotransmitter pathway genes and how they may influence behavioural phenotypes.

Keywords: dopamine; serotonin; polymorphisms; expression; psychiatry

Introduction

There have been several reviews of the association between polymorphisms of candidate genes and

behavioural traits and disorders (Baron, 1999; Wong et al., 2000; Catalano, 2001; McAllister and Summerall, 2003; Ueno, 2003). Although some of the variants studied may affect gene function, many are anonymous. The purpose of this chapter is to focus attention on the evidence supporting the potential functional significance of polymorphisms in neurotransmitter metabolism genes and their

*Corresponding author. Tel.: +44(0) 207-848-0741;
Fax: +44(0) 207-848-0866

possible role in behavioural disorders. Given the practicalities of attempting to annotate adequately the review and given our recent coverage of the material (D'Souza and Craig, 2006), we will concentrate our bibliography on important developments concerning the significant impact of functional polymorphisms in behavioural disorders. To this end, we have included not only variation within coding regions, but also that located in upstream promoter sequences, introns and 5' and 3' transcribed, but untranslated regions (5' and 3'UTRs). Functional variation is most often represented by single nucleotide polymorphisms (SNP) or tandem repeat sequences. Copy number changes in these latter motifs, particularly, can be important in controlling the levels and patterns of gene expression and may consequently play a significant role in the aetiology of quantitative behavioural traits. In order to evaluate the possible significance of such potentially functional variation in behavioural dimensions, we will focus on evidence for variation in genes of the dopamine and serotonin pathways.

The evidence available is based on a variety of approaches. These include transient transfection methods with mammalian cell lines employing constructs which couple the sequences of interest to reporter gene assays. More recently, comparisons have been made between gene expression levels from cells or tissues from individuals with specific genotypes which, additionally, may enable the significance of environment effects to be evaluated. For some genes, in vivo expression patterns have been compared employing functional brain imaging signals. Pharmacological ligand binding and neuroimaging studies have also provided a direct assessment of functional effects of polymorphisms in coding regions of neurotransmitter pathway genes. Finally, the use of transgenic mice has provided an alternative strategy to obtain in vivo evidence for the functional effects of polymorphisms in regulatory regions.

In this chapter, we review the potential significance of SNPs and simple microsatellites in and around the coding regions of the following well-annotated genes in the dopamine and serotonin pathways: dopamine receptor D₃ (*DRD3*), dopamine receptor D₄ (*DRD4*), *DAT1* (*SLC6A3*), tyrosine hydroxylase (*TH*), catechol-*O*-methyltransferase

(*COMT*), brain-derived neurotrophic factor (*BDNF*), 5-hydroxytryptamine receptor 2A (*HTR2A*), monoamine oxidase A (*MAOA*) and *SERT* (*5-HTT*, *SLC6A4*) (see Tables 1 and 2 for gene nomenclature, chromosome location, OMIM accession numbers and description of polymorphisms). Furthermore, because of the potential functional significance of complex repeating motifs, we additionally focus on the information currently available concerning the effects of such motifs on gene regulation and their reported possible behavioural consequences.

Dopamine receptor D₃, *DRD3*

A *BalI/MscI* (p.Ser9Gly) restriction fragment length polymorphism is located at amino acid position 9 of the N-terminal extra-cellular domain of the *DRD3*, which corresponds to a point mutation in the first coding exon of the gene (Lannfelt et al., 1992). The Ser9 variant is referred as allele 1 and the Gly9 variant referred as allele 2. Significant associations ($P < 0.002$) between the 1–1 genotype and schizophrenia have been reported in case-control studies in French and English populations (Crocq et al., 1992; Mant et al., 1994; Asherson et al., 1996; Spurlock et al., 1998b). A meta-analysis of case-control studies also was in agreement with these previous findings ($P = 0.004$) (Williams et al., 1998). Additionally, a further meta-analysis found an excess of the 1–1 genotype in schizophrenics in African and Caucasian groups at $P < 0.05$ (Dubertret et al., 1998). Interestingly, this mutation results in a slightly higher affinity for agonist binding compared to the wild-type receptor when expressed in recombinant cell lines (Lundstrom and Turpin, 1996). In contrast, more recent evidence fails to support association of this polymorphism with schizophrenia (Anney et al., 2002; Jonsson et al., 2004; Staddon et al., 2005).

Dopamine receptor D₄, *DRD4*

A polymorphism (c.–521C>T) has been identified in the promoter region of the dopamine D₄ receptor gene and has been reported to be weakly

Table 1. List of OMIM accession numbers for the genes described and their association with disorders

Gene name	OMIM accession number	Chromosome location	Polymorphisms reported for gene	Behavioural disorders possibly associated
<i>DRD3</i>	126451	3q13.3	p.Ser9Gly	Schizophrenia
<i>DRD4</i>	126452	11p15.5	c.-521C>T g.120bpdup 48 bp VNTR	Schizophrenia, ADHD, methamphetamine abuse
<i>DAT1, SLC6A3</i>	126455	5p15.3	3'UTR VNTR Int8VNTR	ADHD ADHD, cocaine abuse
<i>TH</i>	191290	11p15.5	VNTR termed as HUMTH01	Schizophrenia
<i>COMT</i>	116790	22q11.2	p.Val158Met	Schizophrenia, schizoaffective disorder, suicide
<i>BDNF</i>	113505	11p13	p.Val66Met	Major depression, bipolar disorder
<i>HTR2A</i>	182135	13q14-q21	c.102T>C c.-1438A>G p.Ile197Val	Schizophrenia
<i>MAOA</i>	309850	Xp11.23	uVNTR	Aggression, antisocial behaviour
<i>SERT, 5-HTT, SLC6A4</i>	182138	17q11.1-q12	5-HTTLPR Intron 2 VNTR, also termed as Stin2 VNTR	Affective disorders

associated ($P = 0.02$) with schizophrenia. This variant showed some functional effects when tested in cells (Okuyama et al., 2000). Transient transfection methods employing the chloramphenicol acetyltransferase (CAT) reporter gene assay revealed the T allele to have a 40% reduction in transcriptional activity compared with the C allele in Y-79 cells. However, more recently, this polymorphism was found to have no significant effect on transcriptional activity in Y-79 cells or in neuroblastoma cell lines (SK-N-F1, IMR32) (Kereszturi et al., 2006). There are several differences in the experimental design between the two studies that could explain the discrepancy in results. These include application of different reporter vector systems and variation in the length of the promoter fragment examined.

One of the most extensively studied coding region variants at this locus is a 48 bp repeat in exon 3 of the *D₄* dopamine receptor gene which codes for the third cytoplasmic loop of this G-protein-coupled receptor. The 48 bp sequence exists as two-, four- or seven-repeat sequences and several studies have demonstrated association and/or linkage between the seven-repeat allele in *DRD4* and attention deficit hyperactivity disorder (ADHD) generally in the significance range of $P = 0.01$ – 0.001 (LaHoste et al., 1996; Rowe et al., 1998; Smalley et al., 1998;

Swanson et al., 1998; Faraone et al., 1999, 2001; Holmes et al., 2000; Tahir et al., 2000; Mill et al., 2001; Gornick et al., 2007). Although there are negative findings, the weight of evidence provided from a recent meta-analysis is in favour of a strong association between ADHD and the *DRD4* seven-repeat allele (Li et al., 2006). Further studies are needed to clarify the precise nature of the variant of *DRD4* or of a nearby gene which accounts for this association (Faraone et al., 2001). Initially, it was claimed that the long form (seven-repeat allele) had different binding profiles with antagonists (clozapine and spiperone) with respect to sodium chloride sensitivity as compared to the short forms (Van Tol et al., 1992). Later, however, the same authors found that the copy number had generally little influence on *DRD4* pharmacological properties although the potency of dopamine to inhibit the stimulation of cyclic AMP (cAMP) levels by forskolin was about twofold reduced for the seven-repeat compared with the two- and four-repeat variants (Asghari et al., 1994, 1995). The exon 3 region of *DRD4* is also capable of coupling to several G-protein (*G_{iα}*) subtypes, but no quantitative differences related to the number of repeats have been detected (Kazmi et al., 2000). It is interesting that in addition to the 48 bp copy number polymorphism, additional diversity also

Table 2. List of GenBank accession numbers and locations of frequent polymorphic markers relative to the genes described

Gene	Mutations (p: protein, c: coding region, g: genomic region)	GenBank, accession number	Version number	rs (dbSNP) numbers
<i>DRD3</i>	p.Ser9Gly	U32499	U32499.1, GI:927341	rs6280
<i>DRD4</i>	c.-521C>T	U95122	U95122.1, GI:2228761	rs12720366
	g.120bpdup	U95122	U95122.1, GI:2228761	
<i>DAT1, SLC6A3</i>	48 bp VNTR (exon 3)	L12398,X58497	L12398.1, GI:291945	
	3'UTR VNTR (exon 15)	NM_001044	NM_001044.2, GI:38194225	
	Int8 VNTR	NM_001044	NM_001044.3, GI:133908627	
<i>TH</i>	VNTR termed as HUMTH01 (intron 1)	M23597	M23597.1, GI:986907	
	p.Val81Met (exon 2)	NM_199292	NM_199292.2, GI:88900500	
<i>COMT</i>	p.Val158Met	Z26491	Z26491.1, GI:403303	
<i>BDNF</i>	p.Val66Met	X91251	X91251.1, GI:987871	rs6265
<i>HTR2A</i>	c.T102C	NM_000621	NM_000621.2, GI:60302916	
	c.-1438A>G	S78723	S78723.1, GI:1042173	
	p.Ile197Val	NM_000621	NM_000621.2, GI:60302916	
<i>MAOA</i>	UVNTR	M89636	M89636.1, GI:187356	
	c.C936T (exon 8)	NM_000240, XM_499175	NM_000240.2, GI:33469954	
<i>5HTT</i>	5-HTTLPR	X76753	X76753.2, GI:4894173	
	A/G SNP in 5-HTTLPR	AB031251	AB031251.1, GI:6863000	rs25531
	Intron 2 VNTR (Stin2 VNTR)	AB031254	AB031254.1, GI:6863003	
		NT_010641	NT_010641.15, GI:51474120	

results from sequence variation (SNP) (Litcher et al., 1993; Ding et al., 2002). The potential functional impact of this has yet to be explored in detail.

Promoter and silencer motifs have been characterized in the 5'flanking region of the *DRD4* gene (Kamakura et al., 1997). Subsequently, a repeat polymorphism in the 5'regulatory region of the *DRD4* gene was identified (Seaman et al., 1999). The variation was found to exist as a tandem duplication of 120 bp (g.120bpdup) located 1.2kb upstream from the initiation codon and approximately 850 bp upstream of the start of transcription and upstream from the previously identified promoter region. The recent frequency estimates of the duplicated allele range from 0.4 to 0.9 in 33 populations around the world. There are consensus-binding sequences for several transcription factors within the duplication suggesting that this polymorphism may have a role in regulating the transcriptional activity. Interestingly, a report was published which showed strong association of this tandem duplication with children having ADHD with the inattentive phenotypic subtype ($P = 0.005$) and it was hypothesized that the duplication could be a risk factor by decreasing expression of the *DRD4* gene (McCracken et al., 2000). Transient transfections in human neuroblastoma and other cell lines with coupled luciferase reporter gene assays have demonstrated that the duplication had lower transcriptional activity in SK-N-MC, SH-SY5Y, HEK293 and HeLa cells (D'Souza et al., 2004). Recently, this result has been replicated by Kereszturi et al. (2007) who showed the tandem duplication together with three SNPs formed a haplotype in the 5'region of *DRD4* which had significantly lower transcriptional activity in Y-79, SK-N-F1 and HeLa cells than the non-duplicated form. Interestingly, they also identified a novel four-repeat allele of the 120 bp duplication and this together as a haplotype with the SNP markers showed significantly lower transcriptional activity than constructs having the one- and two-repeat alleles of the 120 bp duplication polymorphism.

There is further evidence of association of the 120 bp duplication on its own ($P = 0.006$) (Kustanovich et al., 2004), or together with a

haplotype of markers in the 5'end and/or exon 3 of the gene with ADHD (Barr et al., 2001a, $P = 0.048$; Mill et al., 2003, $P = 0.03$; Arcos-Burgos et al., 2004, $P = 0.0467$). Additionally, associations of this repeat have been reported with schizophrenia ($P = 0.003$) (Xing et al., 2003) and (less robustly) with methamphetamine abuse ($P = 0.01$) (Li et al., 2004). Other studies have reported association to novelty seeking in bipolar ($P = 0.01$) and alcoholic families ($P = 0.006$) (Rogers et al., 2004). It is also worth mentioning that no evidence of the duplication polymorphism was found with ADHD in a Taiwanese population (Brookes et al., 2005) and in an Indian population (Bhaduri et al., 2006).

Dopamine transporter, *DAT1*, *SLC6A3*

The dopamine transporter is a 620-amino acid protein belonging to the family of Na^+/Cl^- -dependent neurotransmitter transporters with 12 putative trans-membrane domains located on the pre-synaptic membrane of nerve terminals. It plays a key role in regulating dopamine neurotransmission by mediating the active uptake of dopamine from the synapse. The gene is located on chromosome 5 (5p15.3) consisting of 15 exons and spans 60 kb. Exon 15 of the gene includes a stop codon, 3'UTR and polyadenylation signal. The 3'UTR contains a VNTR with copies of a 40 bp tandem repeat unit ranging from 3 to 11 with the 9 and 10 repeats being the most common alleles (Vandenberg et al., 1992). Studies with this marker led to the implication of *DAT1* in ADHD, with the first genetic study showing a significant association between the disorder and the 10-repeat allele ($P = 0.006$) (Cook et al., 1995). Subsequently, several research groups replicated this finding in different clinical samples with overall P -values ranging from 0.01 to 0.001 (Gill et al., 1997; Waldman et al., 1998; Daly et al., 1999; Barr et al., 2001b; Curran et al., 2001; Chen et al., 2003). Faraone et al. (2005) have provided an evaluation of the evidence for the association of the 3'UTR with ADHD and, on the basis of the pooled odds ratio (OR) across studies, found a small, but statistically significant OR in favour

of this association. Recently, a meta-analysis of results from transmission disequilibrium testing of the 10-repeat allele of the polymorphism showed a small but significant association with ADHD (Yang et al., 2007). Because many mRNAs contain 3'UTR elements that bind regulatory proteins and form RNA-protein complexes that control mRNA transport, translation and stability (Mignone et al., 2002; Wickens et al., 2002; Kuersten and Goodwin, 2003), it is reasonable to anticipate that the 3' polymorphism in the *DAT1* gene may have functional effects.

Several studies have investigated this hypothesis using cell line and reporter gene assay methodologies (Fuke et al., 2001; Michelhaugh et al., 2001; Inoue-Murayama et al., 2002; Miller and Madras, 2002; Greenwood and Kelsoe, 2003; Mill et al., 2005). However, these studies have generated conflicting results largely due to employment of different cloning strategies and constructs, which do not mirror the 3'UTR location of the polymorphism in vivo. Additionally, different viral promoters have been employed and previous evidence has shown variation in transcriptional activity provided by these promoters (Zarrin et al., 1999). Furthermore, different cell lines were used which may not express all the transcription factors necessary for regulation of the *DAT1* gene. The first study showed the 10-repeat allele of *DAT1* to have higher luciferase expression than the 7- or 9-repeat alleles in COS-7 cells (derived from African Green Monkey kidney) and also in human glioblastoma A172 cells (Fuke et al., 2001). These constructs carried the repeat alleles downstream of the luciferase gene and upstream of the polyadenylation signal and also included the cytomegalovirus (CMV) promoter. A later study revealed the nine-repeat allele to enhance transcription in SN4741 cells (a mouse embryonic substantia nigra-derived cell line) also within dopaminergic neurons in neonatal rat midbrain slices (Michelhaugh et al., 2001). In this investigation, the nine-repeat allele was inserted upstream of the simian virus 40 (SV40) promoter and green fluorescent protein (GFP) used as the reporter gene. However, this study did not investigate the functional effects of the 10-repeat allele, but nevertheless suggests that the VNTR polymorphism may play a role in the

regulation of the *DAT1* gene. Interestingly, they found that the nine-repeat allele interacted with proteins from whole cell extracts prepared from embryonic stem cells using the electrophoretic mobility shift assay (EMSA). Binding was disrupted by increasing amounts of DAT VNTR probe and also by probe derived from the serotonin transporter VNTR (see below for description of this polymorphism). Miller and Madras (2002) subsequently demonstrated that the 9-repeat alleles in both humans and rhesus monkeys increased *DAT1* expression in HEK293 cells relative to 10-repeat alleles, when inserted downstream of the luciferase gene and in the presence of herpes simplex virus thymidine kinase (HSV-TK) and SV-40 promoters in the constructs. However, in these studies, expression was further mediated by the identification of a SNP also located in the 3'UTR region but also downstream of the VNTR. A more recent study demonstrated vectors with the endogenous *DAT1* promoter and the 9-repeat alleles of the 3'UTR VNTR showed significantly higher levels of luciferase activity than vectors having the 10-repeat alleles in SH-SY5Y cells (Fuke et al., 2005). Further reports including one from our laboratory have revealed no significant differences in transcription between the 9- and 10-repeat alleles when cloned downstream of the luciferase gene and in the presence of either the SV40 or the *DAT1* homologous promoters in SK-N-SH, SN4741, HEK293 or SH-SY5Y cells (Inoue-Murayama et al., 2002; Greenwood and Kelsoe, 2003; Fuke et al., 2005; Mill et al., 2005).

Previous ex vivo expression studies from our laboratory have investigated the functional effects of the VNTR polymorphism with the 3'UTR of the *DAT1* gene where the mRNA levels were measured in post-mortem brain tissue (cerebellum and temporal lobe) and lymphocytes using a semi-quantitative real-time reverse transcription polymerase chain reaction method (Mill et al., 2002). The results revealed that in both post-mortem brain tissue and peripheral blood lymphocytes, *DAT1* expression was found to be significantly higher in individuals with the 10-repeat allele compared to the 9-repeat alleles. A recent report from our laboratory has replicated this finding and showed in post-mortem midbrain tissue that

higher DAT1 gene expression is significantly correlated with the 10-repeat allele of the 3'UTR polymorphism compared to the 9-repeat allele (Brookes et al., 2007).

Thus, although it seems clear that the 3' VNTR has a role in regulating expression, there are discrepancies in determining the differential effects of the various alleles. In particular, the interpretation of results from transfection studies is complicated by lack of information concerning the exact role of the 3'UTR. To date, the most direct method for examining its effects is by measuring mRNA levels directly on normal cells or tissues from individuals carrying different genotypes. Given the inconsistencies in the data obtained from in vitro studies, an in vivo approach using transgenic mice may provide a more robust, direct approach to the characterization of the mechanisms of *DAT1* transcriptional regulation.

Neuroimaging approaches have also been employed to study this polymorphism; however, to date only small and non-reproducible differences resulting from genotype effects of the 9 and/or 10 repeats have been observed (Heinz et al., 2000a; Jacobsen et al., 2000; Martinez et al., 2001).

VanNess et al. (2005) performed DAT1-binding assays and Western blot experiments and showed that the 10-repeat allele of the VNTR had higher affinity for its substrate and higher DAT1 density than the 9-repeat allele. These data are in agreement with the expression studies ex vivo and some of the in vitro findings. Another VNTR was identified in the *DAT1* gene which is located within intron 8 (Int8 VNTR) and found to be associated with ADHD in both English and Taiwanese samples (Brookes et al., 2006) and with cocaine abuse in a Brazilian sample (Guindalini et al., 2006). Furthermore, in the ADHD study, there was evidence of a specific haplotype effect, with only the haplotype consisting of the 10-repeat allele of the 3'UTR VNTR and the 3-repeat allele of the Int8 VNTR showing association with ADHD. Functional studies of the VNTR in intron 8 were carried out using reporter constructs transfected in mouse dopaminergic cells (SN4741 cell line). These cells when in a normal or 'resting' state showed the three-repeat allele to confer slightly less expression of the reporter construct

than the two-repeat allele. This reduction in expression was increased by 40% when cocaine was added to the culture medium (Guindalini et al., 2006). Interestingly, the 3-repeat allele either on its own or in the presence of the 10-repeat allele of the 3'UTR VNTR was found to correlate with increased levels of the *DAT1* transcript in mid-brain post-mortem tissue (Brookes et al., 2006). These findings emphasize the differences between in vitro and ex vivo studies.

Tyrosine hydroxylase, TH

A potentially functional SNP, Val81Met, has been recognized in exon 2 of this gene (Lüdecke and Bartholomé, 1995) and has been studied in context of alcoholism with a positive association observed for early onset (Dahmen et al., 2005), but not for adult classifications of this disorder (Ishiguro et al., 1998).

In addition to locations in coding regions, 5' and 3' flanking regions, potentially functional tandem repeats have also been detected within introns. One such example is the tetranucleotide repeat polymorphism located in the first intron of the *TH* gene (Polymeropoulos et al., 1991). The gene encodes the rate-limiting enzyme in the synthesis of catecholamines. The first intron harbours the HUMTH01 microsatellite that has 5–10 repetitions of the core motif TCAT. The 10-repeat allele exhibits two sequence variants: a (TCAT)₄CAT (TCAT)₅ imperfect repeat (allele T10i) which is frequent in the Caucasian general population (>30%) and a perfect repeat (TCAT)₁₀ (allele T10p) which is rare (~1%) and reported to be significantly associated with schizophrenia in a French population ($P < 0.01$) (Meloni et al., 1995). In functional studies, the 10-repeat alleles were cloned upstream of the thymidine kinase minimal promoter and exhibited enhancer function using the luciferase reporter gene in expression vectors and transient transfection in HeLa cells, PC12 (cells derived from rat pheochromocytoma) and SK-N-SH (from human neuroblastoma tumours) (Meloni et al., 1998). Moreover, the repeated sequences bound to specific protein complexes

from HeLa cell nuclear extracts belonging to the Fos–Jun family of proteins using EMSAs.

Later functional studies from the same laboratory reported the quantitative silencing effects of the TCAT repeat on *TH* gene expression in two catecholaminergic cell lines, PC12 and CHP212 (human neuroblastoma) when the first intron of the *TH* gene (including the promoter region) was inserted in frame with the luciferase gene, mimicking its *in vivo* location. In contrast to the previous observations, the 10-repeat alleles showed the highest inhibition of transcriptional activity compared with 3- or 5-repeat alleles. The observed effects were suggested to be due to the different positions of the (TCAT) repeat with respect to the promoter (Albanese et al., 2001). Additionally, Albanese et al. (2001) showed that the (TCAT)_n repeat interacts with HBP1 (a HMG box transcription factor) and ZNF191 (a zinc finger protein) using the yeast hybrid method. More recently, chromatin immune precipitation (ChIP) with antibodies directed against HBP1 was performed to ascertain the implication of this repeated sequence on the expression of the *TH* gene ‘*in vivo*’ and to identify other genes that could be regulated by this factor (Meloni et al., 2007). The quantitative PCR analysis of the ChIP immune-precipitated genomic DNA confirmed binding ‘*in vivo*’ of HBP1 to the HUMTH01 microsatellite in the *TH* gene. Additionally, about 200 genes were identified from the ChIP products and a preliminary computational ‘*in silico*’ analysis revealed that several of these genes belong to different pathways that may contribute to regulation of neurotransmission.

Recent detailed studies of the transcriptional regulatory properties of the promoter, first exon and first intron employing cell lines permissive and non-permissive for *TH* expression indicated the presence of repressor elements (–6244/–194) with enhancing elements within the first intron (+730/+1653) (Romano et al., 2007). Other studies further indicate a complex interaction of elements regulating *TH* expression, including the transcription factors Pitx3 and Nurr1 (Messmer et al., 2007), GATA-3/CREB (Hong et al., 2006) and the androgen receptor complex (Jeong et al., 2006). This information coupled with the

revelation of additional potential regulatory tissue-specific methylation of the first exon (Aranyi et al., 2005) provides an insight into the complexity of the situation and suggests simple functional interpretation of polymorphic variants at this locus should be undertaken with caution.

Catechol-*O*-methyltransferase, COMT

The COMT gene encodes an enzyme that is involved in the metabolism of catecholamine neurotransmitters. It exists in soluble and membrane forms and it is well established that a common functional SNP (G to A transition at codon 158) within the coding region of the gene results in a valine-to-methionine substitution (Val158Met) that is associated with a three- to fourfold reduction in the biochemical activity of the COMT enzyme and increased thermolability. There are also numerous reports linking this polymorphism to a range of behaviours including psychosis and violent behaviour (Lotta et al., 1995). There is evidence that the low-activity Met158 allele may be associated with violent behaviour among patients with schizophrenia and schizoaffective disorder (Strous et al., 1997, 2003; Lachman et al., 1998; Kotler et al., 1999, with *P*-values of 0.005, 0.005, 0.02 and 0.003, respectively). A similar observation has been made with violent suicide attempters (*P* = 0.04) (Rujescu et al., 2003). In addition, a three-marker haplotype of the *COMT* gene (including the Val allele of Val158Met polymorphism) was found to be very significantly associated with schizophrenia in a large sample of Ashkenazi Jews with a *P*-value of 9.5×10^{-8} (Shifman et al., 2002).

Both neuroimaging and pharmacogenomic studies have analysed the function of the Val158Met SNP. Functional MRI approaches have examined the effect of this polymorphism, revealing that the Met allele (which encodes the enzyme with lower activity) is associated with better performance tests of prefrontal cortex function (executive function and working memory) and prefrontal cortex physiology (Egan et al., 2001; Goldberg et al., 2003; Mattay et al., 2003). Additionally, evidence has revealed that adrenocorticotrophin (ACTH)

and cortisol responses to naloxone (opiate antagonist) were higher in subjects with Met/Met genotype compared with subjects homozygous or heterozygous for the Val allele (Oswald et al., 2004). It was concluded from these findings that individual differences in catecholamine metabolism resulting from *COMT* variants could influence hypothalamic-pituitary-adrenal axis function and play a pharmacogenetic role in response to naloxone. Furthermore, after administration of antipsychotics such as olanzapine, schizophrenic patients with the Met allele displayed enhanced working memory performance and prefrontal cortex activation (Bertolino et al., 2004; Weickert et al., 2004). Thus, overall, the balance of evidence is in favour of the interpretation of the effect of the Val/Met polymorphism with the original observations of Egan et al. (2001).

A number of studies have additionally determined the effects of this SNP on gene expression levels. First, an investigation using post-mortem human tissue and in situ hybridization techniques revealed that the Val allele was associated with increased TH expression in mesencephalic dopamine neurons (Akil et al., 2003). Subsequently, Bray et al. (2003) demonstrated lower expression of the Val allele compared with the Met allele in frontal, parietal and temporal cortex post-mortem human brain using a primer extension SnapShot assay to distinguish the transcripts. They additionally found the *COMT* haplotype previously implicated in schizophrenia (Li et al., 1996; Shifman et al., 2002) was associated with lower expression of *COMT* mRNA. Recent findings also demonstrate lower expression of the Val allele compared to the Met allele in both human brain samples and lymphoblast cell lines using a 5' nuclease assay (Zhu et al., 2004). In contrast, a recent study indicated that *COMT* activity is approximately 40% higher in the dorsolateral pre-frontal cortex in human subjects with the *COMT* Val allele than in those with the *COMT* Met allele (Chen et al., 2004a). Unlike Bray et al., they failed to show a differential effect on mRNA levels using real-time quantitative PCR. More recently, Dempster et al. (2006) provided evidence that genotype is related to *COMT* gene expression and that three SNPs, the Val158Met (rs165688-Val

allele), rs737865 (G allele) and rs165599 (G allele), all showed reduced expression. They also demonstrated a strong sexual dimorphism in *COMT* expression, with females exhibiting significantly greater levels of *COMT* mRNA. Interpretations of association are therefore complex and complicated by the possible dual action of the Met substitution affecting both enzyme thermolability and RNA expression in an opposite manner. An indirect effect of functional variation of *COMT* and psychosis has also been observed in context of adolescent exposure to cannabis with the Met allele giving increased predisposition to psychosis in this group. This association was not observed in individuals who commenced exposure to cannabis as adults (Caspi et al., 2005).

More recently, in addition to further reports of associations of the Val158Met polymorphism with a range of affective disorders (e.g. Craddock et al., 2006), two significant developments are of interest. The first concerns its potential role in schizophrenia. In a comprehensive review, Williams et al. (2007) while finding no simple relationship between this polymorphism and the disorder did discover some support for the view that *COMT* influences susceptibility to some forms of psychosis.

There is also new evidence suggesting that the promoter of the membrane-bound form of *COMT* is hypomethylated in autopsy brain samples (particularly the prefrontal lobes) of schizophrenic and bipolar depression patients. Low levels of promoter methylation at CpG sites are frequently associated with increased transcriptional activity and quantitative gene expression studies on corresponding tissue samples demonstrated a correlation between increased transcript levels and hypomethylation in disease patients compared with controls. Of particular interest was the finding that hypomethylation was enriched for patients carrying the Val allele (Abdolmaleky et al., 2006). It appeared that the methylation level was associated with the Val/Met polymorphism at one CpG site within the promoter ($P = 0.042$), with Val¹⁵⁸ homozygotes exhibiting lower levels of methylation than Met¹⁵⁸ homozygotes. The same relationship was observed at a second promoter CpG site, although not reaching statistical significance. In addition to suggesting a possible

additional functional effect of the Val/Met polymorphism, it may well be that epigenetic changes may represent one of the complicating factors preventing a simplistic association between *COMT* variants, their impact on the activity of their gene product and these complex disorders.

Brain-derived neurotrophic factor, BDNF

BDNF is known to modulate hippocampal plasticity and hippocampal-dependent memory in cell models and animals. It plays an important role in regulating transmitter systems, neuronal survival and regeneration, and is consequently a key candidate for implication in behavioural disorders. Like other peptide growth factors, the *BDNF* gene encodes a precursor peptide (pro-BDNF) that is proteolytically cleaved to form a mature protein. Interestingly, BDNF has been shown to modulate serotonin transporter function in lymphoblast cells (Mossner et al., 2000) and variants in this locus are highly relevant to the functionality of the monoamine pathways. A SNP has been identified in the 5'pro-BDNF sequence at nucleotide 196 (G/A) producing an amino acid substitution (valine to methionine) at codon 66 (Val66Met). The Met allele has been found to be associated with bipolar disorder in adults ($P = 0.00064$) (Neves-Pereira et al., 2002; Sklar et al., 2002; Lohoff et al., 2005) although a more recent, very large study finds significant association only with a rapidly cycling subset ($P = 0.004$) (Green et al., 2006). It has also been associated with binge eating (Monteleone et al., 2006) and with neuroticism, a known risk factor for depression ($P = 0.0057$) (Sen et al., 2003; Jiang et al., 2005); however, there are also conflicting reports (Willis-Owen et al., 2005). There are also both positive (Schumacher et al., 2005; Hwang et al., 2006) and negative (e.g. Tsai et al., 2003; Surtees et al., 2007) reports on the association of the polymorphism with major depression disorders. It is possible that the association relates to the psychotic features and suicidal behavioural components of this disorder (Iga et al., 2007), which may explain the heterogeneity of the results. Also of interest is the construction of a variant BDNF mouse

reproducing the phenotypic hallmarks of the Met carrying humans (Chen et al., 2006).

A few studies have examined the functional impact of this polymorphism with the first revealing that Met allele exhibited abnormal intracellular trafficking and impaired secretion of BDNF protein compared with those transfected with the Val allele in rat hippocampal neurons (Egan et al., 2003). Furthermore, magnetic resonance spectroscopic imaging studies in humans revealed that the Met allele was associated with poorer episodic memory and abnormal hippocampal activation and lower hippocampal *N*-acetyl aspartate (NAA) (Egan et al., 2003). An additional study investigated the impact of the BDNF polymorphism on memory-related hippocampal activity and found the Met allele to have diminished hippocampal engagement in comparison with Val homozygotes during encoding and retrieval processes using blood oxygen level-dependent (BOLD) fMRI (Hariri et al., 2003). Recent data indicate that the polymorphism results in abnormal trafficking in neuronal cells and when expressed together in the same cell, BDNF_{Met} alters the trafficking of BDNF_{Val} via formation of heterodimers that are less efficiently stored into the regulated secretory pathway (Chen et al., 2004b).

A complex structure embracing three types of dinucleotide repeats which is located about 1.0 kb from the translation initiation site has been investigated. Additional complexity conferred by insertion/deletion and nucleotide substitutions within the repeat motifs resulted in the identification of 23 alleles. One major allele was associated with bipolar disorder ($P = 0.001$) and functional studies in a luciferase-coupled assay suggest this allele conferred reduced transcription (Okada et al., 2006).

In a detailed dissection of the *BDNF* locus, Pruunsild et al. (2007) showed that the gene has 11 exons and 9 functional promoters which operate in a tissue- and brain region-specific manner. A significant role may also be attributed to a range of differentially spliced non-coding antisense transcripts which are produced from an anti-BDNF locus (BDNFOS), which form double-stranded duplicates in the brain in vivo.

Various trans-acting factors may serve to modulate the expression of the locus including the

transcription factor Pitx3 (Peng et al., 2007) and there is evidence that oestrogen may also be important in its regulation (Scharfman and MacLusky, 2006; Sohrabji and Lewis, 2006). It is also of interest that the *BDNF* locus is also one of the few defined targets of the methyl-binding protein *MECP2* gene, mutations in which are responsible for Rett syndrome (Chen et al., 2003). Again the picture emerges of a gene with excellent credentials as a candidate for involvement in a range of behavioural disorders, but also of one whose complexity of regulation and response to external and trans-acting factors complicate simplistic interpretations.

5-Hydroxytryptamine receptor 2A, HTR2A

The C allele of the silent T102C polymorphism in the coding region of the *HTR2A* receptor gene is another coding region SNP found to be significantly associated with schizophrenia (Williams et al., 1996, $P = 0.003$; Spurlock et al., 1998a, $P = 0.006$). Previous negative findings could be due to ethnic differences and a recent meta-analysis of 31 case-control association studies has confirmed the significant association between the C allele and schizophrenia. This was more evident in European samples than in the entire sample ($P < 0.001$) (Abdolmaleky et al., 2004). Differential expression of the two alleles of this *HTR2A* polymorphism was observed using a restriction fragment length polymorphism-based assay. The C allele was found to have intrinsically lower expression than the T allele in the temporal cortex, revealing a possible molecular mechanism contributing to schizophrenia (Polesskaya and Sokolov, 2002). Recently, however, experiments using the more robust primer extension-based assay failed to find this difference in expression in post-mortem cortical brain tissue (Bray et al., 2004). Further studies will be necessary to clarify the possible differential transcriptional status of the two alleles. Furthermore, given the synonymous nature of this coding region SNP, it is likely that any such differences will relate to transcriptional control motifs in linkage disequilibrium.

Additional SNP variants have been identified in the coding region of the *HTR2A* receptor and their respective products tested for response to the antagonists loxapine and clozapine following stable transformation in Sf9 insect cells (Harvey et al., 2003). Only one, Ile197Val, showed statistically significant results. This variant is located in the internal portion of the fourth trans-membrane domain, and required a twofold higher concentration of the atypical neuroleptic drug, clozapine, to inhibit serotonin stimulation compared to the wild-type receptor.

An A1438AG polymorphism was identified which is located close to the promoter region of the *HTR2A* receptor gene and luciferase reporter assays have revealed that both A and G alleles have significant basal promoter activity in HeLa cells (known to express HTR2A) (Spurlock et al., 1998a). However, no significant differences in the basal transcriptional activity existed between the two alleles when constructs contained only the more upstream of the two *HTR2A* promoters. A similar observation was obtained when the promoter activity was induced by cAMP and protein kinase C-dependent mechanisms (Spurlock et al., 1998a). On the other hand, more recent findings using both luciferase and CAT assays with SH-SY5Y and HeLa cells found the transcriptional activity for the G allele to be significantly lower than the A allele when the more downstream promoter was included in the constructs in the presence of an enhancer region (Parsons et al., 2004). There is some evidence of association of this polymorphism with obsessive-compulsive disorder (Walitza et al., 2002) and negative symptom response to olanzapine in schizophrenia (Ellingrod et al., 2003).

Other functional studies with a haplotype of both the c.-1438A>G marker in the regulatory region and the SNP (c.102T>C) located in the coding region described above (Spurlock et al., 1998a) revealed that HTR2A binding (assayed with ^3H ketanserin) in the prefrontal cortex was higher in suicide victims having the haplotype 102T/-1438A compared with non-suicide individuals with the haplotype 102C/-1438G (Turecki et al., 1999). These differences in protein binding are in the same direction as changes in gene expression from our

own findings (Parsons et al., 2004) and in another previous study (Polesskaya and Sokolov, 2002). However, it is important to note that these differences were not observed in other binding studies (Kouzmenko et al., 1997, 1999; Hrdina and Du, 2001). It was suggested that the conflicting results from the ligand-binding studies could be due to the specificity of ketanserin for the 5-HT_{2A} receptor (Parsons et al., 2004). A more recent study by Myers et al. (2007) has revealed that the promoter activity of the *HTR2A* gene is significantly decreased in the presence of both the G-allele of the -1438A/G polymorphism and another SNP in the regulatory region of the gene.

Monoamine oxidase A, MAOA

MAOA is an X-linked gene expressed in the outer mitochondrial membrane of specific cells in various brain and peripheral tissues. The enzyme catalyzes the degradation of several biological amines such as serotonin and norepinephrine. There is strong evidence that MAOA plays an important role in human and animal behaviour. First, a nonsense mutation (in exon 8 of the gene that leads to the production of a truncated, non-functional, protein) was found to be associated with impulsive aggressive behaviour in affected males in a single large family studied in Holland (Brunner et al., 1993). Increased aggression also characterized adult male transgenic mice having a deletion in the *MAOA* gene (Cases et al., 1995). For these reasons, there has been particular interest in examining both the possible function and the behavioural significance of less dramatic variants at this locus. Hotamisligil and Breakefield (1991) provided evidence that population variants, detected by restriction fragment polymorphisms for *EcoRV* and *Fnu4HI*, were associated with altered levels of MAOA activity in male fibroblasts. Although interpretations on activity in cultured skin biopsies are complicated by the presence of non-expressing cells (Denney et al., 1999), it is probable that the results reflect substantial linkage disequilibrium (allelic association) between the RFLPs and functionally significant upstream promoter variants (see below).

In addition to the RFLPs, a variety of microsatellite markers have been detected within and around the *MAOA* loci including a VNTR in intron 1 (Hinds et al., 1992) and an (AC)_n microsatellite in intron 2 of the *MAOA* gene (Black et al., 1991). Several of these have been employed in association studies with a range of behavioural disorders.

Interestingly, although null variants are extremely rare, characterization of the promoter region of the gene revealed a VNTR in the 5' regulatory region of the gene (Zhu et al., 1992; Denney et al., 1994; Zhu and Shih, 1997) which proved to have functional effects. The VNTR polymorphism termed is located 1.2 kb upstream of the coding region and comprises a 30 bp repeated sequence present in 3, 3.5, 4 or 5 copies. Transfection studies with a vector containing the luciferase gene and various human neuroblastoma lines and a human placental choriocarcinoma cell line showed that alleles with 3.5 or 4.0 copies of the repeat sequence are transcribed 2–10 times more efficiently than those with 3 or 5 copies of the repeat (Sabol et al., 1998). This suggested that regulation via repeat length may have phenotypic consequences. Indeed, the longer alleles (3.5, 4 and 5 repeats) were significantly more frequent in females with panic disorder ($P = 0.001$) (Deckert et al., 1999). These authors also carried out functional experiments with luciferase reporter gene constructs transfected into neuroblastoma cells and found that the 3-repeat allele had 1.3–1.8-fold lower transcriptional activity than the alleles having 3.5, 4 and 5 repeats. Additionally, another study using an MAOA enzyme/antibody assay provided evidence that the promoter VNTR repeat number has a similar effect in male skin fibroblast cultures to that observed in transfection studies, with the three-repeat genotype resulting in lower enzyme content than the four-repeat genotype (Denney et al., 1999). Investigations have also determined the effects of the polymorphism in brain post-mortem brain tissue (Balciuniene et al., 2002). They observed a slight tendency for higher MAOA enzyme activity for individuals carrying four repeats of the VNTR. A particular combination of polymorphisms for the VNTR and a SNP in the nearby *MAOB* gene, however, revealed significant

lower MAOA enzyme levels in the brain suggesting that there may be another unknown functional polymorphism in linkage disequilibrium with the promoter. A recent study has investigated the regulation of *MAOA* by glucocorticoid and androgen receptors and the transcription factor Sp1 and a novel factor R1 whose interactions overall result in up-regulation by both glucocorticoids and androgens (Ou et al., 2006). Positron emission tomography has been applied to determine MAOA activity in brain of individuals with the long or short alleles using the radioligand ^{11}C -chlorglyline and in contrast to transfection and cell line studies failed to detect significant differences in activity levels (Fowler et al., 2007).

Intriguingly, VNTRs are also observed in the promoter regions of *MAOA* of a range of apes (Wendland et al., 2005); however, intra-species differences in transcriptional activity dependent on copy number of these motifs have not generally been observed (Inoue-Murayama et al., 2006).

One of the most studied aspects of the significance of functional variants in *MAOA* is that relating to antisocial behaviour (ASB) and aggression in males. Simple association studies on the relationship between high- and low-activity promoter VNTR alleles and ASB have been rather inconsistent, but with an apparent trend for associations with the low-activity variants, particularly with aggression related to alcohol abuse. Conversely, there are several reports of internalizing disorders including anxiety, neuroticism and panic disorder to be associated with the high-activity variants (reviewed in Craig, 2005).

Perhaps of more robust significance, however, have been the observations that the environment can be a major determinant concerning the behavioural outcomes of different *MAOA* promoter variants. In a study on gene–environment effects, maltreated children having the genotype in the 5' regulatory region that confers high levels of *MAOA* expression were found to be less likely to develop antisocial problems (Caspi et al., 2002). Some subsequent attempts to replicate these observations have been supportive of a significant role for *MAOA* variants interacting with stressful

upbringing in predisposing ASB (Foley et al., 2004; Nilsson et al., 2006) and as predictors of destructive behaviour during male adolescent alcohol consumption (Nilsson et al., 2007). Although Haberstick et al. (2005) failed to demonstrate a significant interaction of low MAOA interaction with maltreatment (assessed retrospectively), they did observe a non-significant trend for low-MAOA-activity individuals to exhibit ASB if they experienced 'severe' levels of victimization. However, in subsequent investigations, Huizinga et al. (2006) were unable to find evidence that MAOA moderates the relationship between adolescent maltreatment and ASB. Young et al. (2006) were also unable to detect a significant genetic–environmental interaction with *MAOA* genotype for maltreatment. They examined a mixed sample of adolescents selected for persistent conduct and substance abuse with no control group for comparison and the study was therefore atypical in this regard. In an attempt to address the heterogeneity in observations, Kim-Cohen et al. (2006) undertook both replication experiments and a meta-analysis of the relationship between *MAOA* genotype, maltreatment and gene-by-environment interaction, in predicting children's mental health. This has provided further support for the importance of *MAOA* genotype in influencing the vulnerability to environmental stress (particularly that encountered in childhood and adolescence) in the predisposition to ASB outcomes.

There are far fewer data relating to MAOA functional variants and females, possibly resulting from the complication of its X-localization and the perceived potential confound of its undetermined level of escape from inactivation; however, in a recent study, a $G \times E$ interaction was observed — suggesting that girls with low-activity alleles may be at increased risk of criminal behaviour in the presence of psychosocial risk. A recent study of allelic expression patterns of *MAOA* in brain has concluded that there was no evidence for skewing in normal individuals (Pinsonneault et al., 2006). The interrelationship between the functional variants in the *MAOA* promoter and stress HPA axis stress response which may underpin the $G \times E$ interaction has been recently reviewed (Craig, 2007).

Serotonin transporter, SERT, 5-HTT, SLC6A4

The most studied polymorphism of this *5HTT* locus is located 1 kb upstream from the transcription start site and identified initially by Heils et al. (1995, 1996). The basic polymorphism (*5-HTTLPR*) comprises a 44 bp insertion or deletion, creating either a 'long' 16-repeat allele or a 'short' 14-repeat allele together with other low-frequency alleles including 15, 18, 19, 20 and 22 repeats with various additional SNPs distinguishing some repeats (Delbruck et al., 1997; Kunugi et al., 1997; Michaelovsky et al., 1999; Nakamura et al., 2000).

Now classic studies (reviewed in D'Souza and Craig, 2006) have suggested that the short allele is associated both with lower transcriptional potential and with anxiety-related personality traits particularly neuroticism and affective disorders. Functional differences between the short and the long alleles studied by genotypic correlation and by expression of reporter gene constructs have been reported in mammalian cell lines, lymphocytes, blood platelets, blood and brain. The first indication that the long variant of the *5-HTTLPR* had higher transcriptional efficiencies than the short variant arose from studies on constructs in which it was fused to the luciferase gene and transfected into human placental choriocarcinoma (JAR) cells that constitutively express *5-HTT* (Heils et al., 1996). Additionally, the same authors reported that the short allele had lower transcriptional activity, decreased 5-HTT expression and decreased 5-HT uptake in peripheral lymphoblasts (Lesch et al., 1996). The pattern has also been supported in part by neuroimaging studies. A variety of subsequent studies have generally supported the higher transcriptional levels observed for the long allele, but the picture is complicated by the studies of SNPs both within the promoter repeats and elsewhere in the gene. A G/A polymorphism has been reported to convert the activity associated with the long allele to that expected for the short allele with the so-called L_G allele behaving in a similar manner to the S allele (Hu et al., 2006). These authors also showed an association of the 'gain of function' high-activity L_A allele with obsessive compulsive disorder

(OCD). Although the allele frequency for L_G is low (10–15% in Caucasians), the observations may subtly alter the interpretations of previous association studies based on a simple L vs. S allele dichotomy; in addition, a further SNP in the repeat region (rs25531) may further complicate the interpretation.

In a more recent investigation of the potential influence of SNPs on transcription, Martin et al. (2007) employed allele expression imbalance methodology to study the influence of 55 SNPs in a region of 100 kb embracing the locus. They were able to confirm the greater expression of the L as opposed to the S form but were unable to confirm the effect of an A>G substitution in the promoter repeat (although this may be a result of inadequate power or effects of other functional variants in the gene). In this regard, it is of considerable importance that they detected significant correlations with allelic transcript imbalance of two of the ranges of SNPs studied. The most significant effect is associated with the genotype of rs16965628, located in the middle of the first intron. The second SNP (rs 2020933) showing a significant effect on transcription is closely associated with the first ($r^2 = 0.79$) and also located in intron 1, but neither SNP is strongly associated with the *5-HTTLPR*. Because of this, it will be important to correct for potential effects of the two intron 1 SNPs when considering potential associations of the *5-HTTLPR* genotype on behavioural disorders. Indeed, when the proportion of total variance in allele expression imbalance is estimated, about 70% is accounted for by the two SNPs together with the promoter polymorphism.

A number of investigations have examined the functional effects of this polymorphism in human post-mortem brain tissue. Initially, using quantitative autoradiographic and in situ hybridization methods in midbrain samples that contain the dorsal and median raphe nuclei, the serotonin transporter mRNA levels and serotonin transporter binding were significantly higher in subjects with the long allele compared to those with either the short allele or heterozygotes. Furthermore, the serotonin transporter-binding sites in subjects with either short or mixed genotype were found to be significantly higher in ethanol-user subjects than

controls (Little et al., 1998). However, other studies failed to detect any relationship between the *5-HTTLPR* genotype and the affinity and/or density of 5-HT binding to human post-mortem brain tissue (Naylor et al., 1998; Mann et al., 2000).

Neuroimaging involving a SPECT study with (I-123) β -CIT reported a higher in vivo 5-HTT protein availability in healthy l/l individuals relative to l/s and s/s genotypes (Heinz et al., 2000b). However, this observation was not replicated in a study of the midbrain of healthy subjects employing the same radioligand (Willeit et al., 2001). Magnetic resonance imaging studies have revealed individuals with one or two copies of the short allele to exhibit greater amygdala neuronal activity in response to fearful stimuli compared with individuals homozygous for the long allele when assessed by BOLD functional magnetic resonance imaging (Hariri et al., 2002). A subsequent study reported that reduced hippocampal volumes were associated with the long variant of the serotonin transporter polymorphism in patients with major depression (Frodl et al., 2004).

It is apparent that both association and functional studies have been difficult to replicate consistently. Another confounding factor which may lead to non-replication of association studies with the promoter variants may be a failure to control for environmental effects. The importance of this has been well illustrated by a study of a gene-by-environment interaction which showed that individuals having one or two copies of the short allele of the *5-HTTLPR* polymorphism exhibited more depressive symptoms, diagnosable depression and suicidality in relation to stressful life events (threat, loss, humiliation or defeat) than individuals homozygous for the long allele (Caspi et al., 2003). A second study demonstrated that individuals with adolescent depression had a significant interaction between environmental risk with short alleles of *5-HTTLPR* and the effect was observed in females only (Eley et al., 2004). Such observations are highly reminiscent of the pattern of gene-by-environment effects observed for the *MAOA* promoter variants and aggression described above. Additionally, an independent group have replicated (Kaufman et al., 2004) the

main observations of Caspi et al. (2003) and further replication has been documented for adults by Kendler et al. (2005) and Wilhelm et al. (2006) although Gillespie et al. (2005) were unable to reproduce the gene-by-environment effect. A recent study of 1913 Caucasian individuals recruited by the Collaborative Study on the Genetics of Alcoholism (COGA) including 881 with life-time depression symptomatology and 679 with alcohol dependence found a significant association of the short allele of the *5HTTLPR* employing a pedigree disequilibrium test with life-time depression in the full sample ($P = 0.02$) with over transmission to affected offspring. Even greater significance was found when the analysis was confined to depressed individuals who had experienced stressful life events. No significant association was observed for those who had not experienced stress (Dick et al., 2007). Overall, the original observations of Caspi et al. appear to have been replicated robustly.

As reviewed previously (D'Souza and Craig, 2006), in addition to the promoter repeat, another example of a functional VNTR polymorphism in the serotonin transporter gene is located in intron 2. It comprises 9, 10 or 12 copies of a 16/17bp element. The functional effects of this specific intronic polymorphism, termed as Stin2 VNTR, have been examined through a series of expression studies. The 10- and 12-repeat alleles were cloned in a luciferase gene-coupled expression vector and transfected the constructs into embryonic stem cells, HM-1. The 12-repeat allele was found to be a stronger enhancer than the 10-repeat allele suggesting that the motif may act as a transcriptional regulator in differentiating embryonic stem cells (Fiskerstrand et al., 1999). These results were in agreement with findings from an in vivo study using transgenic mice, where the 10 and 12 alleles were cloned into an expression vector containing a heterologous minimal promoter and the bacterial LacZ reporter gene and then injected into fertilized eggs (MacKenzie and Quinn, 1999). At embryonic day E10.5, the 12 allele showed significantly stronger levels of β -galactosidase expression in the developing rostral hindbrain compared with the 10 allele. The data suggest that the VNTR region acts as a transcriptional regulator in an

allele-dependent manner with the 12-repeat allele having stronger enhancer properties than the 10-repeat allele. Additional studies suggest that not only the number, but also the primary structure of the repeats could affect the transcription of the gene (Lovejoy et al., 2003). Furthermore, two transcription factors, Y box-binding protein 1 (YB-1) and CTCF-binding factor (CTCF), were found to be responsible for modulation of Stin2 VNTR function as a transcriptional regulatory domain (Klenova et al., 2004).

Indeed, given the possibility that the *5-HTT* locus may be a target for the therapeutic effects of lithium (LiCl) in treatment of depression, Roberts et al. (2007) have examined the potential interaction of the Stin2 polymorphism with this agent. It appears that LiCl modified the levels of CTCF and YB-1 mRNA and protein and that both CTCF and YB-1 showed differential binding to the allelic variants of the intron 2 VNTR and may result in alterations to the expression of *SLC6A4*. The complexity of the interactions is further increased by the potential interaction of LiCl with the *5-HTTLPR* which contains domains potentially capable of interaction with CTCF.

At the pharmacogenetic level, it has been noted that individuals homozygous for the 12-repeat allele were found to have lower affinity of 5-HT uptake than individuals who had the 10/9 genotype (Kaiser et al., 2002). The vast literature on the clinical response to selective serotonin reuptake inhibitors (SSRIs) in depressive patients with different serotonin transporter genotypes has been reviewed recently (Smiths et al., 2004). The evidence points to a weighted response rate of 36% for the 10/12 genotype and of 80% for the 12/12 genotype in Asians only ($P < 0.001$). These indications of functional significance may underlie reported associations with behavioural disorders including the strong evidence for increased frequency of allele 12 of the VNTR with subjects having bipolar disorder ($P = 0.00048$) (Collier et al., 1996). Additionally, significant differences exist between patients with unipolar disorder and controls in the proportion of individuals having allele 9 (Battersby et al., 1996; Ogilvie et al., 1996). Finally, an increased frequency of the 10 allele of this intron polymorphism together with the

long allele of the promoter polymorphism of the serotonin transporter gene was observed in a suicide cohort having Slavic ethnicity ($P = 0.0112$) (Hranilovic et al., 2003). Thus, most of the evidence suggests that this polymorphism is functional and may influence affective disorders, but there is a need to determine the exact nature of the correlation between the VNTR copy number with depressive phenotype and the potential interaction with the promoter variant (*5-HTTLPR*) as the association with depression has not always been reproducible. Lastly, an independent report revealed that the combined genotype at both *5-HTTLPR* and Stin2 locus ('low-expressing' genotypes containing at least short allele) reduced mRNA expression levels in lymphoblastoid cell lines from a sample of individuals with schizophrenia or schizoaffective disorder using a real-time PCR method (Hranilovic et al., 2004).

Conclusion

The literature provides a wealth of data underlining the importance of both SNP and micro-satellite/VNTR variants in modulating the functionality of genes in the dopamine and serotonin pathways and the possibility that such variation may be likely to be significant in the aetiology of normal and abnormal behaviours will continue to intrigue researchers. Their endeavours will be assisted by continued research and clarification of the functional impact of the genetic repertoire of these neurotransmitter pathway genes. It will therefore be necessary to determine the nature of the underlying molecular mechanisms at work. It is known that genes acting in the same genetic, biochemical or physiological process are often regulated co-ordinately by specific combinations of transcription factors that bind to explicit sites in DNA. Transcription factors interact with each other when they bind to their target sites and such protein-DNA complexes (referred as transcriptosomes) are responsible for the combinational transcriptional regulation of the genes.

Preliminary analysis reveals multiple transcription factor-binding sites as a common feature of

promoters of the set of genes in the dopamine and serotonin pathway along with their mouse and rat homologues. This raises the intriguing possibility that the coordinated control of these pathways may be amenable to detailed investigations of the regulatory motifs including some of the types we have reviewed in this chapter.

Acknowledgements

We acknowledge the Medical Research Council (MRC) who supported research on a component grant entitled 'Functional analysis of mutations in genes of monoamine neurotransmitters metabolism that are associated with behavioural disorders' (Grant No.: #G0000197). We thank Kelley Halton for secretarial support especially with formatting of the text and references.

References

- Abdolmaleky, H.M., Cheng, K.H., Faraone, S.V., Wilcox, M., Glatt, S.J., Gao, F., Smith, C.L., Shafa, R., Aali, B., Carnevale, J., Pan, H., Papageorgis, P., Ponte, J.F., Sivaraman, V., Tsuang, M.T. and Thiagalingam, S. (2006) Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Hum. Mol. Genet.*, 15: 3132–3145.
- Abdolmaleky, H.M., Faraone, S.V., Glatt, S.J. and Tsuang, M.T. (2004) Meta-analysis of association between the T102C polymorphism of the 5HT2a receptor gene and schizophrenia. *Schizophr. Res.*, 67: 53–62.
- Akil, M., Kolachana, B.S., Rothmond, D.A., Hyde, T.M., Weinberger, D.R. and Kleinman, J.E. (2003) Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *J. Neurosci.*, 23: 2008–2013.
- Albanese, V., Biguet, N.F., Kiefer, H., Bayard, E., Mallet, J. and Meloni, R. (2001) Quantitative effects on gene silencing by allelic variation at a tetranucleotide microsatellite. *Hum. Mol. Genet.*, 10: 1785–1792.
- Anney, R.J., Rees, M.I., Bryan, E., Spurlock, G., Williams, N., Norton, N., Williams, H., Cardno, A., Zammit, S., Jones, S., Jones, G., Hoogendoorn, B., Smith, K., Hamshire, M.L., Coleman, S., Guy, C., O'Donovan, M.C., Owen, M.J. and Buckland, P.R. (2002) Characterisation, mutation detection, and association analysis of alternative promoters and 5' UTRs of the human dopamine D3 receptor gene in schizophrenia. *Mol. Psychiatry*, 7: 493–502.
- Aranyi, T., Fauchoux, B.A., Khalfallah, O., Vodjdani, G., Biguet, N.F., Mallet, J. and Meloni, R. (2005) The tissue-specific methylation of the human tyrosine hydroxylase gene reveals new regulatory elements in the first exon. *J. Neurochem.*, 94: 129–139.
- Arcos-Burgos, M., Lopera, F., Rapoport, J.L., Pineda, D., Castellanos, F.X., Konecki, D., Bailey-Wilson, J., Palacio, J.D. and Berg, K. (2004) Pedigree disequilibrium test (PDT) replicates association and linkage between DRD4 and ADHD in multigenerational and extended pedigrees from a genetic isolate. *Mol. Psychiatry*, 9: 252–259.
- Asghari, V., Sanyal, S., Buchwaldt, S., Paterson, A., Jovanovic, V. and Van Tol, H.H. (1995) Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J. Neurochem.*, 65: 1157–1165.
- Asghari, V., Schoots, O., Van Kats, S., Ohara, K., Jovanovic, V., Guan, H.-C., Bunzow, J.R., Petronis, A. and Van Tol, H.H.M. (1994) Dopamine D₄ receptor repeat: analysis of different native and mutant forms of the human and rat genes. *Mol. Pharmacol.*, 46: 364–373.
- Asherson, P., Mant, R., Holmans, P., Williams, J., Cardno, A., Murphy, K., Jones, L., Collier, D., McGuffin, P. and Oweb, M.J. (1996) Linkage, association and mutational analysis of the dopamine D₃ receptor gene in schizophrenia. *Mol. Psychiatry*, 1: 125–132.
- Balciuniene, J., Emilson, L., Orelund, L., Pettersson, U. and Jazin, E.E. (2002) Investigation of the functional effect of monoamine oxidase polymorphisms in human brain. *Hum. Genet.*, 110: 1–7.
- Baron, M. (1999) Candidate genes and behavioural traits — candidly. *Arch. Gen. Psychiatry*, 56: 582–583.
- Barr, C.L., Feng, Y., Wigg, K.G., Schachar, R., Tannock, R., Roberts, W., Malone, M. and Kennedy, J.L. (2001a) 5'Untranslated region of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Am. J. Med. Genet B Neuropsychiatr. Genet.*, 105B: 84–90.
- Barr, C.L., Xu, C., Kroft, J., Feng, Y., Wigg, K., Zai, G., Tannock, R., Schachar, R., Malone, M., Roberts, W., Nothen, M.M., Grunhage, F., Vanderbergh, D.J., Uhl, G., Sunohara, G., King, N. and Kennedy, J.L. (2001b) Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyperactivity disorder. *Biol. Psychiatry*, 49: 333–339.
- Battersby, S., Ogilvie, A.D., Smith, C.A., Blackwood, D.H., Muir, W.J., Quinn, J.P., Fink, G., Goodwin, G.M. and Harmor, A.J. (1996) Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. *Psychiatr. Genet.*, 6: 177–181.
- Bertolino, A., Caforio, G., Blasi, G., De Candia, M., Latorre, V., Petruzzella, V., Altamura, M., Nappi, G., Papa, S., Callicot, J.H. and Mattay, V.S. (2004) Interaction of COMT Val^{108/158} Met genotype and olanzapine treatment on prefrontal cortical function in patients with schizophrenia. *Am. J. Psychiatry*, 161: 1798–1805.
- Bhaduri, N., Das, M., Sinha, S., Chattopadhyay, A., Gangopadhyay, P.K., Chaudhuri, K., Singh, M. and Mukhopadhyay, K. (2006) Association of dopamine D4 receptor (DRD4) polymorphisms with attention deficit hyperactivity disorder in Indian population. *Am. J. Hum. Genet.*, 141: 61–66.

- Black, G.C., Chen, Z.Y., Craig, I.W. and Powell, J.F. (1991) Dinucleotide repeat polymorphism at the MAOA locus. *Nucleic Acid Res.*, 19: p. 689.
- Bray, N.J., Buckland, P.R., Hall, H., Owen, M.J. and O'Donovan, M.C. (2004) The serotonin-2A receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. *Mol. Psychiatry*, 9: 109–114.
- Bray, N.J., Buckland, P.R., Williams, N.M., Williams, H.J., Norton, N., Owen, M.J. and O'Donovan, M.C. (2003) A haplotype implicated in schizophrenia susceptibility is associated with reduced COMT expression in human brain. *Am. J. Hum. Genet.*, 73: 152–161.
- Brookes, K.J., Mill, J., Guindalini, C., Curran, S., Xu, X., Knight, J., Chen, C.K., Huang, Y.S., Sethna, V. and Taylor, E. (2006) A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol pregnancy. *Arch. Gen. Psychiatry*, 63: 74–81.
- Brookes, K.J., Neale, B.M., Sugden, K., Khan, N., Asherson, A. and D'Souza, U.M. (2007) Relationship between VNTR polymorphisms of the human dopamine transporter gene and expression in post-mortem midbrain tissue. *Am. J. Med. Genet B Neuropsychiatr. Genet.*, 144B: 1070–1078.
- Brookes, K.J., Xu, X., Chen, C.-K., Huang, Y.-S., Wu, Y.-Y. and Asherson, P. (2005) No evidence for the association of DRD4 with ADHD in a Taiwanese population within-family study. *BMC Med. Genet.*, 6: p. 31.
- Brunner, H.G., Nelen, M., Breakefield, X.O., Ropers, H.H. and van Oost, B.A. (1993) Abnormal behaviour associated with a point mutation in the structural gene for monoamine oxidase A. *Science*, 262: 578–580.
- Cases, O., Seif, I., Grimsby, J., Gaspar, P., Chen, K., Pournin, S., Muller, U., Aguet, M., Babinet, C., Shih, J.C., et al. (1995) Aggressive behaviour and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. *Science*, 270: 362–364.
- Caspi, A., McClay, J., Moffitt, T.E., Mill, J., Martin, J., Craig, I.W., Taylor, A. and Poulton, R. (2002) Role of genotype in the cycle of violence in maltreated children. *Science*, 297: 851–854.
- Caspi, A., Moffitt, T.E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R. and Craig, I.W. (2005) Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-*O*-methyltransferase gene: longitudinal evidence of a gene \times environment interaction. *Biol. Psychiatry*, 57: 1117–1127.
- Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. and Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, 301: 386–389.
- Catalano, M. (2001) Functionally gene-linked polymorphic regions and genetically controlled neurotransmitters metabolism. *Eur. Neuropsychopharmacol.*, 11: 431–439.
- Chen, C.-K., Chen, S.-L., Mill, J., Huang, Y.-S., Lin, S.-K., Curran, S., Purcell, S., Sham, P. and Asherson, P. (2003) The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. *Mol. Psychiatry*, 8: 393–396.
- Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., Egan, M.F., Kleinman, J.E. and Weinberger, D.R. (2004a) Functional analysis of genetic variation in catechol-*O*-methyltransferase (COMT): effects on mRNA, protein and enzyme activity in post-mortem human brain. *Am. J. Hum. Genet.*, 75: 807–821.
- Chen, Z.-Y., Jing, D., Bath, K.G., Ieraci, A., Khan, T., Siao, C.-J., Herrera, D.G., Toth, M., Yang, C., McEwen, B.S., Hempstead, B.L. and Lee, F.S. (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behaviour. *Science*, 314: 140–143.
- Chen, Z.-Y., Patel, P.D., Sant, G., Meng, C.-X., Teng, K.K., Hempstead, B.L. and Lee, F.S. (2004b) Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J. Neurosci.*, 24: 4401–4411.
- Collier, D.A., Arranz, M.J., Sham, P., Battersby, S., Vallada, H., Gill, P., Aitchison, K.J., Sodhi, M., Li, T., Roberts, G.W., Smith, B., Morton, J., Murray, R.M., Smith, D. and Kirov, G. (1996) The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. *Neuroreport*, 7: 1675–1679.
- Cook, E.H., Stein, M.A., Krasowski, M.D., Cox, N.J., Olkon, D.M., Kieffer, J.E. and Leventhal, B.L. (1995) Association of attention-deficit disorder and the dopamine transporter gene. *Am. J. Hum. Genet.*, 56: 993–998.
- Craddock, N., Owen, M.J. and O'Donovan, M.C. (2006) The catechol-*O*-methyltransferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. *Mol. Psychiatry*, 11: 446–458.
- Craig, I.W. (2005) The role of monoamine oxidase A, MAOA, in the aetiology of antisocial behaviour; the importance of gene environment interactions. *Novartis Found. Symp.*, 268: 227–237.
- Craig, I.W. (2007) The importance of stress and genetic variation in human aggression. *Bioessays*, 29: 227–236.
- Crocq, M.A., Mant, R., Asherson, P., Williams, J., Hode, Y., Mayerova, A., Collier, D., Lannfelt, L., Sokoloff, P., Schwartz, J.C., Gill, M., Macher, J.P., McGuffin, P. and Owen, M.J. (1992) Association between schizophrenia and homozygosity at the dopamine D₃ receptor gene. *J. Med. Genet.*, 29: 858–860.
- Curran, S., Mill, J., Tahir, E., Kent, L., Richards, S., Gould, A., Hockett, L., Sharp, J., Batten, C., Fernando, S., Ozbay, F., Yazgan, Y., Simonoff, E., Thompson, M., Taylor, E. and Asherson, P. (2001) Association study of a dopamine transporter polymorphism and attention deficit hyperactivity disorder in UK and Turkish samples. *Mol. Psychiatry*, 6: 425–428.

- Dahmen, N., Volp, M., Singer, P., Hiemke, C. and Szegedi, A. (2005) Tyrosine hydroxylase Val-81-Met polymorphism associated with early-onset alcoholism. *Psychiatr. Genet.*, 15: 13–16.
- Daly, G., Hawi, Z., Fitzgerald, M. and Gill, M. (1999) Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT, DBH and DRD5 to affected children. *Mol. Psychiatry*, 4: 192–196.
- Deckert, J., Catalano, M., Syagailo, Y.V., Bosi, M., Okladnova, O., Di Bella, D., Nothen, M.M., Maffei, P., Franke, P., Fritze, J., Maier, W., Propping, P., Beckmann, H., Bellodi, L. and Lesch, K.-P. (1999) Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. *Hum. Mol. Genet.*, 8: 621–624.
- Delbruck, S.J., Wendel, B., Grunewald, I., Sander, T., Morris-Rosendahl, D., Crocq, M.A., Berrettini, W.H. and Hoehe, M.R. (1997) A novel allelic variant of the human serotonin transporter gene regulatory polymorphism. *Cytogenet. Cell Genet.*, 79: 214–220.
- Dempster, E.L., Mill, J., Craig, I.W. and Collier, D.A. (2006) The quantification of COMT mRNA in post mortem cerebellum tissue: diagnosis, genotype, methylation and expression. *BMC Med. Genet.*, 7: p. 10.
- Denney, R.M., Koch, H. and Craig, I.W. (1999) Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promoter-associated variable number tandem repeat. *Hum. Genet.*, 105: 542–551.
- Denney, R.M., Sharma, A., Dave, S.K. and Waguespack, A. (1994) A new look at the promoter of the human monoamine oxidase A gene: mapping transcription initiation sites and capacity to drive luciferase expression. *J. Neurochem.*, 63: 843–856.
- Dick, D.M., Plunkett, J., Hamlin, D., Nurnberger, J., Jr., Kuperman, S., Schuckit, M., Hesselbrock, V., Edenberg, H. and Bierut, L. (2007) Association analyses of the serotonin transporter gene with lifetime depression and alcohol dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) sample. *Psychiatr. Genet.*, 17: 35–38.
- Ding, Y.C., Chi, H.C., Grady, D.L., Morishima, A., Kidd, J.R., Kidd, K.K., Flodman, P., Spence, M.A., Schuck, S., Swanson, J.M. and Moyzis, R.K. (2002) Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proc. Natl. Acad. Sci. U.S.A.*, 99: 309–314.
- D'Souza, U.M. and Craig, I.W. (2006) Functional polymorphisms in dopamine and serotonin pathway genes. *Hum. Mutat.*, 27: 1–13.
- D'Souza, U.M., Russ, C., Tahir, E., Mill, J., McGuffin, P., Asherson, P.J. and Craig, I.W. (2004) Functional effects of a tandem duplication polymorphism in the 5' flanking region of the DRD4 gene. *Biol. Psychiatry*, 56: 691–697.
- Dubertret, C., Gorwood, P., Ades, J., Feingold, J., Schwartz, J.-C. and Sokoloff, P. (1998) Meta-analysis of DRD3 gene and schizophrenia: ethnic heterogeneity and significant association in Caucasians. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 81B: 318–322.
- Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D. and Weinberger, D.R. (2001) Effect of COMT Val^{108/158} Met genotype on frontal lobe function and risk for schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.*, 98: 6917–6922.
- Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B. and Weinberger, D.R. (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112: 257–269.
- Eley, T.C., Sugden, K., Corsico, A., Gregory, A.M., Sham, P., McGuffin, P., Plomin, R. and Craig, I.W. (2004) Gene-environment interaction analysis of serotonin system markers with adolescent depression. *Mol. Psychiatry*, 9: 908–915.
- Ellingrod, V.L., Lund, B.C., Perry, P.J., Bever-Stille, K., Fleming, F., Holman, T.L. and Miller, D. (2003) 5-HT2A receptor promoter polymorphism, -1438G/A and negative symptom response to olanzapine in schizophrenia. *Psychopharmacol. Bull.*, 37: 109–112.
- Faraone, S.V., Biederman, J., Weiffenbach, B., Keith, T., Chu, M.P., Weaver, A., Spencer, T.J., Wilens, T.E., Frazier, J., Cleves, M. and Sakai, J. (1999) Dopamine D₄ gene 7-repeat allele and attention deficit hyperactivity disorder. *Am. J. Psychiatry*, 156: 768–770.
- Faraone, S.V., Doyle, A.E., Mick, E. and Biederman, J. (2001) Meta-analysis of the association between the 7-repeat allele of the dopamine D₄ receptor gene and attention deficit hyperactivity disorder. *Am. J. Psychiatry*, 158: 1052–1057.
- Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A. and Sklar, P. (2005) Molecular genetics of attention-deficit/hyperactivity disorder. *Biol. Psychiatry*, 57: 1313–1323.
- Fiskerstrand, C.E., Lovejoy, E.A. and Quinn, J.P. (1999) An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. *FEBS Lett.*, 458: 171–174.
- Foley, D.L., Eaves, L.J., Wormley, B., Silberg, J.L., Maes, H.H., et al. (2004) Childhood adversity, monoamine oxidase A genotype, and risk for conduct disorder. *Arch. Gen. Psychiatry*, 61: 738–744.
- Frodl, T., Meisenzahl, E.M., Zill, P., Baghai, T., Rujescu, D., Leinsinger, G., Bottlender, R., Schule, C., Zwanzger, P., Engel, R.R., Rupprecht, R., Bondy, B., Reiser, M. and Moller, H.J. (2004) Reduced hippocampal volumes associated with the long variant of the serotonin transporter polymorphism in major depression. *Arch. Gen. Psychiatry*, 61: 177–183.
- Fowler, J.S., Alia-Klein, N., Kriplani, A., Logan, J., Williams, B., Zhu, W., Craig, I.W., Telang, F., Goldstein, R., Volkow, N.D., Vaska, P. and Wang, G.-W. (2007) Evidence that brain MAO A activity does not correspond to MAO A genotype in healthy male subjects. *Biol. Psychiatry*, 62: 355–358.
- Fuke, S., Sasagawa, N. and Ishiura, S. (2005) Identification and characterization of the Hes1/Hey1 as a candidate

- trans-acting factor on gene expression through the 3'non-coding polymorphic region of the human dopamine transporter (DAT1) gene. *J. Biochem.*, 137: 205–216.
- Fuke, S., Suo, S., Takahashi, N., Koike, H., Sasagawa, N. and Ishiura, S. (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics J.*, 1: 152–156.
- Gill, M., Daly, G., Heron, S., Hawi, Z. and Fitzgerald, M. (1997) Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. *Mol. Psychiatry*, 2: 311–313.
- Gillespie, N.A., Whitfield, J.B., Williams, B., Heath, A.C. and Martin, N.G. (2005) The relationship between stressful life events, the serotonin transporter (5-HTTLPR) genotype and major depression. *Psychol. Med.*, 35: 101–111.
- Goldberg, T.E., Egan, M.F., Gscheidle, T., Coppola, R., Weickert, T., Kolachana, B.S., Goldman, D. and Weinberger, D.R. (2003) Executive subprocesses in working memory: relationship to catechol-*O*-methyltransferase Val158Met genotype and schizophrenia. *Arch. Gen. Psychiatry*, 60: 889–896.
- Gornick, M.C., Addington, A., Shaw, P., Bobb, A.J., Sharp, W., Greenstein, D., Arepalli, S., Castellanos, F.X. and Rapoport, J.C. (2007) Association of the dopamine receptor D4 (DRD4) gene 7-repeat allele with children with attention-deficit/hyperactivity disorder (ADHD): an update. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 144B: 379–382.
- Green, E.K., Raybould, R., Macgregor, S., Hyde, S., Young, A.H., O'Donovan, M.C., Owen, M.J., Kirov, G., Jones, L., Jones, I. and Craddock, N. (2006) Genetic variation of brain-derived neurotrophic factor (BDNF) in bipolar disorder: case-control study of over 3000 individuals from the UK. *Br. J. Psychiatry*, 188: 21–25.
- Greenwood, T.A. and Kelsoe, J.R. (2003) Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. *Genomics*, 82: 511–519.
- Guindalini, C., Howard, M., Haddley, K., Laranjeira, R., Collier, D., Ammar, N., Craig, I., O'Gara, C., Bubb, V.J., Greenwood, T., et al. (2006) A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. *Proc. Natl. Acad. Sci. U.S.A.*, 103: 4552–4557.
- Haberstick, B.C., Lessem, J.M., Hopfer, C.J., Smolen, A., Ehringer, M.A., Timberlake, D. and Hewitt, J.K. (2005) Monoamine oxidase A (MAOA) and antisocial behaviors in the presence of childhood and adolescent maltreatment. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 135: 59–64.
- Hariri, A., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F. and Weinberger, D.R. (2002) Serotonin transporter genetic variation and the response of the human amygdala. *Science*, 297: 400–403.
- Hariri, A.R., Goldberg, T.E., Mattay, V.S., Kolachana, B.S., Callicott, J.H., Egan, M.F. and Weinberger, D.R. (2003) Brain-derived neurotrophic factor val⁶⁶met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J. Neurosci.*, 23: 6690–6694.
- Harvey, L., Reid, R.E., Caixia, M., Knight, P.J.K., Pfeifer, T.A. and Grigliatti, T.A. (2003) Human genetic variations in the 5HT2A receptor: a single nucleotide polymorphism identified with altered response to clozapine. *Pharmacogenetics*, 13: 107–118.
- Heils, A., Teufel, A., Petri, S., Seemann, M., Bengel, D., Balling, U., Riederer, P. and Lesch, K.-P. (1995) Functional promoter and polyadenylation site mapping of the human serotonin (5-HT) transporter gene. *J. Neural Transm. Gen. Sect.*, 102: 247–254.
- Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D. and Lesch, K.P. (1996) Allelic variation of human serotonin transporter. *J. Neurochem.*, 66: 2621–2624.
- Heinz, A., Goldman, D., Jones, D.W., Palmour, R., Hommer, D., Gorey, J.G., Lee, K.S., Linnoila, M. and Weinberger, D.R. (2000a) Genotype influences in vivo dopamine transporter availability in human striatum. *Neuropsychopharmacology*, 22: 133–139.
- Heinz, A., Jones, D.W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., Linnoila, M. and Weinberger, D.R. (2000b) A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biol. Psychiatry*, 47: 643–649.
- Hinds, H.L., Hendriks, R.W., Craig, I.W. and Chen, Z.Y. (1992) Characterization of a highly polymorphic region near the first exon of the human MAOA gene containing a GT dinucleotide and a novel VNTR motif. *Genomics*, 13: 896–897.
- Holmes, J., Payton, A., Barrett, J.H., Hever, T., Fitzpatrick, H., Trumper, A.L., Harrington, R., McGuffin, P., Owen, M., Ollier, W., Worthington, J. and Thapar, A. (2000) A family-based and case-control study of the dopamine D₄ receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. *Mol. Psychiatry*, 5: 523–530.
- Hong, S.J., Huh, Y., Chae, H., Hong, S., Lardaro, T. and Kim, K.S. (2006) GATA-3 regulates the transcriptional activity of tyrosine hydroxylase by interacting with CREB. *J. Neurochem.*, 98: 773–781.
- Hotamisligil, G.S. and Breakefield, X.O. (1991) Human monoamine oxidase A gene determines levels of enzyme activity. *Am. J. Hum. Genet.*, 49: 383–392.
- Hranilovic, D., Stefulj, J., Furac, I., Kubat, M., Balija, M. and Jernej, B. (2003) Serotonin transporter gene promoter (5-HTTLPR) and intron 2 (VNTR) polymorphism in Croatian suicide victims. *Biol. Psychiatry*, 54: 884–889.
- Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B. and Wildenauer, D. (2004) Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. *Biol. Psychiatry*, 55: 1090–1094.
- Hrdina, P.D. and Du, L. (2001) Levels of serotonin receptor 2A higher in suicide victims? *Am. J. Psychiatry*, 158: 147–148.
- Hu, X., Lipsky, R.H., Zhu, G., Akhtar, L.A., Taubman, J., Greenberg, B.D., Xu, K., Arnold, P.D., Richter, M.A., Kennedy, J.L., Murphy, D.L. and Goldman, D. (2006) Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am. J. Hum. Genet.*, 78: 815–826.

- Huizinga, D., Haberstick, B.C., Smolen, A., Menard, S., Young, S.E., Corley, R.P., Stallings, M.C., Grotzinger, J. and Hewitt, J.K. (2006) Childhood maltreatment, subsequent antisocial behavior, and the role of monoamine oxidase A genotype. *Biol. Psychiatry*, 60: 677–683.
- Hwang, J.P., Tsai, S.J., Hong, C.J., Yang, C.H., Lin, J.F. and Yang, Y.M. (2006) The Val66Met polymorphism of the brain derived neurotrophic factor gene is associated with geriatric depression. *Neurobiol. Aging*, 27: 1834–1837.
- Iga, J., Shu-Ichi, U., Yamauchi, K., Numata, S., Tayoshi-Shibuya, S., Kinouchi, S., Nakataki, M., Song, H., Hokoishi, K., Tanabe, H., Sano, A. and Ohmori, T. (2007) The val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with psychotic feature and suicidal behavior in Japanese major depressive patients. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 144B: 1003–1006.
- Inoue-Murayama, M., Adachi, S., Mishima, N., Mitani, H., Takenaka, O., Terao, K., Hayasaka, I., Ito, S. and Murayama, Y. (2002) Variation of variable number of tandem repeat sequences in the 3'-untranslated region of primate dopamine transporter genes that affects reporter gene expression. *Neurosci. Lett.*, 334: 206–210.
- Inoue-Murayama, M., Mishima, N., Hayasaka, I., Ito, S. and Murayama, Y. (2006) Divergence of ape and human monoamine oxidase A gene promoters: comparative analysis of polymorphisms, tandem repeat structures and transcriptional activities on reporter gene expression. *Neurosci. Lett.*, 405: 207–211.
- Ishiguro, H., Arinami, T., Saito, T., Akazawa, S., Enomoto, M., Mitushio, H., Fujishiro, H., Tada, K., Akimoto, Y., Mifune, H., Shiozuka, S., Hamaguchi, H., Toru, M. and Shibuya, H. (1998) Systematic search for variations in the tyrosine hydroxylase gene and their associations with schizophrenia, affective disorders, and alcoholism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 81B: 388–396.
- Jacobsen, L.K., Staley, J.K., Zoghbi, S.S., Seibyl, J.P., Kosten, T.R., Innis, R.B. and Gelernter, J. (2000) Prediction of dopamine transporter binding availability by genotype: a preliminary report. *Am. J. Psychiatry*, 157: 1700–1703.
- Jeong, H., Kim, M.S., Kwon, J., Kim, K.S. and Seol, W. (2006) Regulation of the transcriptional activity of the tyrosine hydroxylase gene by androgen receptor. *Neurosci. Lett.*, 396: 57–61.
- Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., Harris, C.R., Marini, A.M., Enoch, M.A. and Lipsky, R.H. (2005) BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology*, 30: 1353–1361.
- Jonsson, E.G., Kaiser, R., Brockmoller, J., Vishwajit, L., Nimgaonkar, L. and Crocq, M.-A. (2004) Meta-analysis of the dopamine D3 receptor gene (DRD3) Ser9Gly variant and schizophrenia. *Psychiatr. Genet.*, 14: 9–12.
- Kaiser, R., Muller-Oerlinghausen, B., Filler, D., Tremblay, P.-B., Berghofer, A., Roots, I. and Brockmoller, J. (2002) Correlation between serotonin uptake in human blood platelets with the 44-bp polymorphism and the 17-bp variable number of tandem repeat of the serotonin transporter. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 114B: 323–328.
- Kamakura, S., Iwaki, A., Matsumoto, M. and Fukumaki, Y. (1997) Cloning and characterisation of the 5' flanking region of the human dopamine D4 receptor gene. *Biochem. Biophys. Res. Commun.*, 235: 321–326.
- Kaufman, J., Yang, B.-Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J.H. and Gelernter, J. (2004) Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc. Natl. Acad. Sci. U.S.A.*, 101: 17316–17321.
- Kazmi, M.A., Snyder, L.A., Cypess, A.M., Graber, S.G. and Sakmar, T.P. (2000) Selective reconstitution of human D4 dopamine receptor variants with G_i subtypes. *Biochemistry*, 39: 3734–3744.
- Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A. and Riley, B. (2005) The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: a replication. *Arch. Gen. Psychiatry*, 62: 529–535.
- Kereszturi, E., Kiraly, O., Barta, C., Molnar, N., Sasvari-Szekely, M. and Csapo, Z. (2006) No direct effect of the -521 C/T polymorphism in the human dopamine D4 receptor gene promoter on transcriptional activity. *BMC Mol. Biol.*, 7: p. 18.
- Kereszturi, E., Tarnok, Z., Nemoda, Z., Gadoros, J., Kiraly, O., Csapo, Z. and Sasvari-Szekely, M. (2007) Association between the 120-bp duplication of the dopamine D4 receptor gene and attention deficit hyperactivity disorder: genetic and molecular analyses. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 144B: 231–236.
- Kim-Cohen, J., Newcombe, R., Williams, B., Moffitt, T.E., Caspi, A., Taylor, A. and Craig, I.W. (2006) MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis. *Mol. Psychiatry*, 11: 903–913.
- Klenova, E., Scott, A.C., Roberts, J., Shamsuddin, S., Lovejoy, E.A., Bergmann, S., Bubb, V.J., Royer, H.-D. and Quinn, J.P. (2004) YB-1 and CTCF differentially regulate the 5-HTT polymorphic intron 2 enhancer which predisposes to a variety of neurological disorders. *J. Neurosci.*, 24: 5966–5973.
- Kotler, M., Barak, P., Cohen, H., Averbuch, I.E., Grinshpoon, A., Gritsenko, I., Nemanov, L. and Ebstein, R. (1999) Homicidal behavior in schizophrenia associated with a genetic polymorphism determining low catechol-O-methyltransferase (COMT) activity. *Am. J. Med. Genet.*, 88: 628–633.
- Kouzmenko, A.P., Hayes, W.L., Pereira, A.M., Dean, B., Burnet, P.W.J. and Harrison, P.J. (1997) 5-HT_{2A} receptor polymorphism and steady state receptor expression in schizophrenia. *Lancet*, 349: p. 1815.
- Kouzmenko, A.P., Scaffidi, A., Pereira, A.M., Hayes, W.L., Copolov, D.L. and Dean, B. (1999) No correlation between A(-1438)G polymorphism in 5-HT_{2A} receptor gene promoter and the density of frontal cortical 5-HT_{2A} receptors in schizophrenia. *Hum. Hered.*, 49: 103–105.

- Kuersten, S. and Goodwin, E.B. (2003) The power of the 3'UTR: translational control and development. *Nat. Rev. Genet.*, 4: 626–637.
- Kunugi, H., Hattori, M., Kato, T., Tatsumi, M., Sakai, T., Sasaki, T., Hirose, T. and Nanko, S. (1997) Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. *Mol. Psychiatry*, 2: 457–462.
- Kustanovich, V., Ishii, J., Crawford, L., Yang, M., McGough, J.J., McCracken, J.T., Smalley, S.L. and Nelson, S.F. (2004) Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. *Mol. Psychiatry*, 9: 711–717.
- Lachman, H.M., Nolan, K.A., Mohr, P., Saito, T. and Volavka, J. (1998) Association between catechol-*O*-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. *Am. J. Psychiatry*, 155: 835–837.
- LaHoste, G.J., Swanson, J.M., Wigal, S.B., Glabe, C., Wigal, T., King, N. and Kennedy, J.L. (1996) Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol. Psychiatry*, 1: 121–124.
- Lannfelt, L., Sokoloff, P., Matres, M.-P., Pilon, C., Giros, B., Jonsson, E., Sedvall, G. and Schwartz, J.-C. (1992) Amino acid substitution in the dopamine D₃ receptor as a useful polymorphism for investigating psychiatric disorders. *Psychiatr. Genet.*, 2: 249–256.
- Lesch, K.-P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H. and Murphy, D.L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274: 1527–1531.
- Li, D., Sham, P.C., Owen, M.J. and He, L. (2006) Meta-analysis shows significant association between dopamine system and attention deficit hyperactivity disorder (ADHD). *Hum. Mol. Genet.*, 15: 2276–2284.
- Li, T., Chen, C.-K., Hu, X., Ball, D., Lin, S.-K., Chen, W., Sham, P.C., Loh, E.-W., Murray, R.M. and Collier, D.A. (2004) Association analysis of the DRD4 and COMT genes in methamphetamine abuse. *Am. J. Med. Genet.*, 129: 120–124.
- Li, T., Sham, P.C., Vallada, H., Xie, T., Tang, X., Murray, R.M., Liu, X. and Collier, D.A. (1996) Preferential transmission of the high activity allele of COMT in schizophrenia. *Psychiatr. Genet.*, 6: 131–133.
- Litcher, J.B., Barr, C.L., Kennedy, J.L., Van Tol, H.H., Kidd, K.K. and Livak, K.J. (1993) A hypervariable segment in the human dopamine D4 (DRD4) gene. *Hum. Mol. Genet.*, 2: 767–773.
- Little, K.Y., McLaughlin, D.P., Zhang, L., Livermore, C.S., Dalack, G.W., McFinton, P.R., DelProposto, Z.S., Hill, E., Cassin, B.J., Watson, S.J. and Cook, E.H. (1998) Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am. J. Psychiatry*, 155: 207–213.
- Lohoff, F.W., Sander, T., Ferraro, T.N., Dahl, J.P. and Berrettini, W.H. (2005) Confirmation of association between the Val66Met polymorphism in the brain derived neurotrophic factor (BNF) gene and bipolar I disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 139B: 51–53.
- Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I. and Taskinen, J. (1995) Kinetics of human soluble and membrane-bound catechol-*O*-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry*, 34: 4202–4210.
- Lovejoy, E.A., Scott, A.C., Fiskerstrand, C.E., Bubb, V.J. and Quinn, J.P. (2003) The serotonin transporter intronic VNTR enhancer correlated with a predisposition to affective disorders has distinct regulatory elements within the domain based on the primary DNA sequence of the repeat unit. *Eur. J. Neurosci.*, 17: 417–420.
- Lüdecke, B. and Bartholomé, K. (1995) Frequent sequence variant in the human tyrosine hydroxylase gene. *Hum. Genet.*, 95: p. 716.
- Lundstrom, K. and Turpin, M.P. (1996) Proposed schizophrenia-related gene polymorphism: expression of the Ser9Gly mutant human dopamine D₃ receptor with the Semliki Forest virus system. *Biochem. Biophys. Res. Commun.*, 225: 1068–1072.
- MacKenzie, A. and Quinn, J. (1999) A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc. Natl. Acad. Sci. U.S.A.*, 96: 15251–15255.
- Mann, J.J., Huang, Y.-Y., Underwood, M., Kassir, S.A., Oppenheim, S., Kelly, T.M., Dwork, A.J. and Arango, V. (2000) A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch. Gen. Psychiatry*, 57: 729–738.
- Mant, R., Williams, J., Asherson, P., Parfitt, E., McGuffin, P. and Owen, M.J. (1994) Relationship between homozygosity at the dopamine D₃ receptor gene and schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 54B: 21–26.
- Martin, J., Cleak, J., Willis-Owen, S.A., Flint, J. and Shifman, S. (2007) Mapping regulatory variants for the serotonin transporter gene based on allelic expression imbalance. *Mol. Psychiatry*, 12: 421–422.
- Martinez, D., Gelernter, J., Abi-Dargham, A., van Dyck, C.H., Kegeles, L., Innis, R.B. and Laruelle, M. (2001) The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. *Neuropsychopharmacology*, 24: 553–560.
- Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H. and Weinberger, D.R. (2003) Catechol-*O*-methyltransferase val¹⁵⁸-met genotype and individual variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci. U.S.A.*, 100: 6186–6191.
- McAllister, T.W. and Summerall, L. (2003) Genetic polymorphisms in the expression and treatment of neuropsychiatric disorders. *Curr. Psychiatry Rep.*, 5: 400–409.
- McCracken, J.T., Smalley, S.L., McGough, J.J., Crawford, L., Del'Homme, M., Cantor, R.M., Liu, A. and Nelson, S.F. (2000) Evidence for linkage of a tandem duplication

- polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity disorder (ADHD). *Mol. Psychiatry*, 5: 531–536.
- Meloni, R., Albanese, V., Ravassard, P., Treilhou, F. and Mallet, J. (1998) A tetranucleotide polymorphic microsatellite, located in the first intron of the tyrosine hydroxylase gene, acts as a transcription regulatory element in vitro. *Hum. Mol. Genet.*, 7: 423–428.
- Meloni, R., Laurent, C., Campion, D., Ben Hadjali, B., Thibaut, F., Dollfus, S., Petit, M., Samolyk, D., Martinez, M., Poirier, M.-F. and Mallet, J. (1995) A rare allele of a microsatellite located in the tyrosine hydroxylase gene found in schizophrenic patients. *C.R. Acad. Sci. III*, 318: 803–809.
- Meloni, R., Sauty, C., Mallet, J. and Faucon Biguet, N. (2007) The polymorphic (TCAT)_N sequence of the HUMTH01 microsatellite in the tyrosine hydroxylase gene and the regulation of gene expression. *J. Psychopharmacol.*, 21: p. S23.
- Messmer, K., Remington, M.P., Skidmore, F. and Fishman, P.S. (2007) Induction of tyrosine hydroxylase expression by the transcription factor Pitx3. *Int. J. Dev. Neurosci.*, 25: 29–37.
- Michaelovsky, E., Frisch, A., Rockah, R., Peleg, L., Magal, N., Shohat, M. and Weizman, R. (1999) A novel allele in the promoter region of the human serotonin transporter gene. *Mol. Psychiatry*, 4: 97–99.
- Michelhaugh, S.K., Fiskerstrand, C., Lovejoy, E., Bannon, M.J. and Quinn, J.P. (2001) The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. *J. Neurochem.*, 79: 1033–1038.
- Mignone, F., Gissi, C., Liuni, S. and Pesole, G. (2002) Untranslated regions of mRNAs. *Genome Biol.*, 3: reviews, 0004.1–0004.10.
- Mill, J., Asherson, P., Browes, C., D'Souza, U. and Craig, I. (2002) Expression of the dopamine transporter gene is regulated by the 3'UTR VNTR: evidence from brain and lymphocytes using quantitative RT-PCR. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 114B: 975–979.
- Mill, J., Asherson, P., Craig, I. and D'Souza, U.M. (2005) Transient expression analysis of allelic variants of a VNTR in the dopamine transporter gene (DAT1). *BMC Genet.*, 6: p. 3.
- Mill, J., Curran, S., Kent, L., Richards, S., Gould, A., Virdee, V., Hockett, L., Sharp, J., Batten, C., Fernando, S., Simanoff, E., Thompson, M., Zhao, J., Sham, P., Taylor, E. and Asherson, P. (2001) Attention deficit hyperactivity disorder (ADHD) and the dopamine D₄ receptor gene: evidence of association but no linkage in a UK sample. *Mol. Psychiatry*, 6: 40–44.
- Mill, J., Fischer, N., Curran, S., Richards, S., Taylor, E. and Asherson, P. (2003) Polymorphisms in the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *Neuroreport*, 14: 1463–1466.
- Miller, G.M. and Madras, B.K. (2002) Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. *Mol. Psychiatry*, 7: 44–55.
- Monteleone, P., Zanardini, R., Tortorella, A., Gennarelli, M., Castaldo, E., Canestrelli, B. and Maj, M. (2006) The 196G/A (val66met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder. *Neurosci. Lett.*, 406: 133–137.
- Mossner, R., Daniel, S., Albert, D., Heils, A., Okladnova, O., Schmitt, A. and Lesch, K.-P. (2000) Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). *Neurochem. Int.*, 36: 197–202.
- Myers, R.L., Airey, D.C., Manier, D.H., Shelton, R.C. and Sanders-Bush, E. (2007) Polymorphisms in the regulatory region of the human serotonin 5-HT_{2A} receptor gene (HTR2A) influence gene expression. *Biol. Psychiatry*, 61: 167–173.
- Nakamura, M., Ueno, S., Sano, A. and Tanabe, H. (2000) The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol. Psychiatry*, 5: 32–38.
- Naylor, L., Dean, B., Pereira, A., Mackinnon, A., Kouzmenko, A. and Copolov, D. (1998) No association between the serotonin transporter-linked promoter region polymorphism and either schizophrenia or density of the serotonin transporter in human hippocampus. *Mol. Med.*, 4: 671–674.
- Neves-Pereira, M., Mundo, E., Muglia, P., King, N., Macciardi, F. and Kennedy, J.L. (2002) The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am. J. Hum. Genet.*, 71: 651–655.
- Nilsson, K.W., Sjöberg, R.L., Damberg, M., Leppert, J., Ohrvik, J., et al. (2006) Role of monoamine oxidase A genotype and psychosocial factors in male adolescent criminal activity. *Biol. Psychiatry*, 59: 121–127.
- Nilsson, K.W., Sjöberg, R.L., Wargelius, H.L., Leppert, J., Lindstrom, L. and Orelund, L. (2007) The monoamine oxidase A (MAO-A) gene, family function and maltreatment as predictors of destructive behaviour during male adolescent alcohol consumption. *Addiction*, 102: 389–398.
- Ogilvie, A.D., Battersby, S., Bubb, V.J., Fink, G., Harmar, A.J., Goodwin, G. and Smith, C.A. (1996) Polymorphism in serotonin transporter gene associated with susceptibility to major depression. *Lancet*, 347: 731–733.
- Okada, T., Hashimoto, R., Numakawa, T., Iijima, Y., Kosuga, A., Tatsumi, M., Kamijima, K., Kato, T. and Kunugi, H. (2006) A complex polymorphic region in the brain-derived neurotrophic factor (BDNF) gene confers susceptibility to bipolar disorder and affects transcriptional activity. *Mol. Psychiatry*, 11: 695–703.
- Okuyama, Y., Ishiguro, H., Nankai, M., Shibuya, H., Watanabe, A. and Arinami, T. (2000) Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait. *Mol. Psychiatry*, 5: 64–69.
- Oswald, L.M., McCaul, M., Choi, L., Yang, X. and Wand, G.S. (2004) Catechol-O-methyltransferase polymorphism

- alters hypothalamic-pituitary-adrenal axis responses to naloxone: a preliminary report. *Biol. Psychiatry*, 55: 102–105.
- Ou, X.M., Chen, K. and Shih, J.C. (2006) Glucocorticoid and androgen activation of monoamine oxidase A is regulated differently by R1 and Sp1. *J. Biol. Chem.*, 281: 21512–21525.
- Parsons, M.J., D'Souza, U.M., Arranz, M.J., Kerwin, R.W. and Makoff, A.J. (2004) The -1438A/G polymorphism in the 5-hydroxytryptamine type 2A receptor gene affects promoter activity. *Biol. Psychiatry*, 56: 406–410.
- Peng, C., Fan, S., Li, X., Fan, X., Ming, M., Sun, Z. and Le, W. (2007) Overexpression of pitx3 upregulates expression of BDNF and GDNF in SH-SY5Y cells and primary ventral mesencephalic cultures. *FEBS Lett.*, 581: 1357–1361.
- Pinsonneault, J.K., Papp, A.C. and Sandee, W. (2006) Allelic mRNA expression of X-linked monoamine oxidase (MAOA) in human brain: dissection of epigenetic and genetic factors. *Hum. Mol. Genet.*, 15: 2636–2649.
- Polesskaya, O.O. and Sokolov, B.P. (2002) Differential expression of the “C” and “T” alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. *J. Neurosci. Res.*, 67: 812–822.
- Polymeropoulos, M., Xiao, H., Rath, D. and Merrill, C. (1991) Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). *Nucleic Acids Res.*, 19: p. 3753.
- Pruunsild, P., Kazantseva, A., Aid, T., Palm, K. and Timmusk, T. (2007) Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. *Genomics*, 90: 397–406.
- Roberts, J., Scott, A.C., Howard, M.R., Breen, G., Bubb, V.J., Klenova, E. and Quinn, J.P. (2007) Differential regulation of the serotonin transporter gene by lithium is mediated by transcription factors, CCCTC binding protein and Y-box binding protein 1, through the polymorphic intron 2 variable number tandem repeat. *J. Neurosci.*, 27: 2793–2801.
- Rogers, G., Joyce, P., Mulder, R., Sellman, D., Miller, A., Allington, M., Robin, O., Wells, E. and Kennedy, M. (2004) Association of a duplicated repeat polymorphism in the 5'-untranslated region of the DRD4 gene with novelty seeking. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 126B: 95–98.
- Romano, G., Macaluso, M., Lucchetti, C. and Iacovitti, L. (2007) Transcription and epigenetic profile of the promoter, first exon and first intron of the human tyrosine hydroxylase gene. *J. Cell. Physiol.*, 211: 431–438.
- Rowe, D.C., Stever, C., Giedinghagen, L.N., Gard, J.M.C., Cleveland, H.H., Terris, S.T., Mohr, J.H., Sherman, S., Abramowitz, A. and Waldman, I.D. (1998) Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol. Psychiatry*, 3: 419–426.
- Rujescu, D., Giegling, I., Gietl, A., Hartmann, A.M. and Moller, H.-J. (2003) A functional single nucleotide polymorphism (V158M) in the COMT gene is associated with aggressive personality traits. *Biol. Psychiatry*, 54: 34–39.
- Sabol, S.Z., Hu, S. and Hamer, D. (1998) A functional polymorphism in the monoamine oxidase A gene promoter. *Hum. Genet.*, 103: 273–279.
- Scharfman, H.E. and MacLusky, N.J. (2006) Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Front. Neuroendocrinol.*, 27: 415–435.
- Schumacher, J., Jamra, R.A., Becker, T., Ohlraun, S., Klopp, N., Binder, E.B., Schulze, T.G., Deschner, M., Schmal, C., Hofels, S., Zobel, A., Illig, T., Propping, P., Holsboer, F., Rietschel, M., Nothen, M.M. and Cichon, S. (2005) Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biol. Psychiatry*, 58: 307–314.
- Seaman, M.I., Fischer, J.B., Chang, F.-M. and Kidd, K.K. (1999) Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 88B: 705–709.
- Sen, S., Nesse, R.M., Stoltenberg, S.F., Li, S., Gleiberman, L., Chakravarti, A., Weder, A.B. and Burmeister, M. (2003) A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology*, 28: 397–401.
- Shifman, S., Bronstein, M., Sternfeld, M., Pisante-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B. and Darvasi, A. (2002) A highly significant association between a COMT haplotype and schizophrenia. *Am. J. Hum. Genet.*, 71: 1296–1302.
- Sklar, P., Gabriel, S.B., McInnis, M.G., Bennett, P., Lim, Y.-M., Tsan, G., Schaffner, S., Kirov, G., Jones, I., Owen, M., Craddock, N., DePaulo, J.R. and Lander, E.S. (2002) Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. *Mol. Psychiatry*, 7: 579–593.
- Smalley, S.L., Bailey, J.N., Palmer, C.G., Cantwell, D.P., McGough, J.J., Del'Homme, M.A., Asarnow, J.R., Woodward, J.A., Ramsey, C. and Nelson, S.F. (1998) Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. *Mol. Psychiatry*, 3: 427–430.
- Smiths, K.M., Smiths, L.J.M., Schouten, J.S.A.G., Stelma, F.F., Nelemans, P. and Prins, M.H. (2004) Influence of SERTPR and Stin2 in the serotonin transporter gene on the effect of selective serotonin reuptake inhibitors in depression: a systematic review. *Mol. Psychiatry*, 9: 433–441.
- Sohrabji, F. and Lewis, D.K. (2006) Estrogen-BDNF interactions: implications for neurodegenerative diseases. *Front. Neuroendocrinol.*, 27: 404–414.
- Spurlock, G., Heils, A., Holmans, P., Williams, J., D'Souza, U.M., Cardno, A., Murphy, K.C., Jones, L., Buckland, P.R., McGuffin, P., Lesch, K.P. and Owen, M.J. (1998a) A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter. *Mol. Psychiatry*, 3: 42–49.
- Spurlock, G., Williams, J., McGuffin, P., Aschauer, H.N., Lenzinger, E., Fuchs, K., Sieghart, W.C., Mezaros, K., Fathi, N., Laurent, C., Mallet, J., Macciardi, F., Pedrini, S., Gill, M., Hawi, Z., Gibson, S., Jazin, E.E., Yang, H.T., Adolfsson,

- R., Pato, C.N., Dourado, A.M. and Owen, M.J. (1998b) European Multicentre Association Study of Schizophrenia: a study of the DRD2 Ser³¹¹ Cys and DRD3 Ser⁹Gly polymorphisms. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 81B: 24–28.
- Staddon, S., Arranz, M.J., Mancama, D., Perez-Nievas, F., Arrizabalaga, I., Anney, R., Buckland, P., Elkin, A., Osborne, S., Munro, J., Mata, I. and Kerwin, R. (2005) Association between dopamine D3 receptor polymorphisms and schizophrenia in an isolate population. *Schizophr. Res.*, 73: 49–54.
- Strous, R.D., Bark, N., Parsia, S.S., Volavka, J. and Lachman, H.M. (1997) Analysis of a functional catechol-*O*-methyltransferase gene polymorphism in schizophrenia: evidence for association with aggressive and antisocial behaviour. *Psychiatry Res.*, 69: 71–77.
- Strous, R.D., Nolan, K.A., Lapidus, R., Diaz, L., Saito, T. and Lachman, H.M. (2003) Aggressive behavior in schizophrenia is associated with the low enzyme activity COMT polymorphism: a replication study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 1B: 29–43.
- Surtees, P.G., Wainwright, N.W., Willis-Owen, S.A., Sandhu, M.S., Luben, R., Day, N.E. and Flint, J. (2007) No association between the BDNF Val66Met polymorphism and mood status in a non-clinical community sample of 7389 older adults. *J. Psychiatr. Res.*, 41: 404–409.
- Swanson, J.M., Sunohara, G.A., Kennedy, J.L., Regino, R., Fineberg, E., Wigal, T., Lerner, M., Williams, L., Lahoste, G.J. and Wigal, S. (1998) Association of the dopamine receptor D₄ (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol. Psychiatry*, 3: 38–41.
- Tahir, E., Yazgan, Y., Cirakoglu, B., Ozbay, F., Waldman, I. and Asherson, P.J. (2000) Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. *Mol. Psychiatry*, 5: 396–404.
- Tsai, S.-J., Cheng, C.-Y., Yu, Y.W.-Y., Chen, T.-J. and Hong, C.-J. (2003) Association study of a brain-derived neurotrophic factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 123B: 19–22.
- Turecki, G., Briere, R., Dewar, K., Antonetti, T., Lesage, A.D., Seguin, M., Chawky, N., Vanier, C., Alda, M., Joober, R., Benkelfat, C. and Rouleau, G.A. (1999) Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am. J. Psychiatry*, 156: 1456–1458.
- Ueno, S. (2003) Genetic polymorphisms of serotonin and dopamine transporters in mental disorders. *J. Med. Invest.*, 50: 25–31.
- Vandenbergh, D.J., Persico, A.M. and Uhl, G.R. (1992) A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic TaqI RFLPs. *Brain Res. Mol. Brain Res.*, 15: 161–166.
- VanNess, S.H., Owens, M.J. and Kilts, C.D. (2005) The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. *BMC Genet.*, 6: p. 55.
- Van Tol, H.H.M., Wu, C.M., Guan, H.-C., Ohara, K., Bunzow, J.R., Civelli, O., Kennedy, J., Seeman, P., Niznik, H.B. and Jovanoic, V. (1992) Multiple dopamine D4 receptor variants in the human population. *Nature*, 358: 149–152.
- Waldman, I.D., Rowe, D.C., Abramowitz, A., Kozel, S.T., Mohr, J.H., Sherman, S.L., Cleveland, H.H., Sanders, M.L., Gard, J.M.C. and Stever, C. (1998) Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. *Am. J. Hum. Genet.*, 63: 1767–1776.
- Walitza, S., Wewetzer, C., Warnke, A., Gerlach, M., Geller, F., Gerber, G., Gorg, T., Herpertz-Dahlmann, B., Schulz, E., Remschmidt, H., Hebebrand, J. and Hinney, A. (2002) 5-HT_{2A} promoter polymorphism -1438 G/A in children and adolescents with obsessive-compulsive disorders. *Mol. Psychiatry*, 7: 1054–1057.
- Weickert, T.W., Goldberg, T.E., Mishara, A., Apud, J.A., Kolachana, B.S., Egan, M.F. and Wienberger, D.R. (2004) Catechol-*O*-methyltransferase Val^{108/158}Met genotype predicts working memory response to antipsychotic medications. *Biol. Psychiatry*, 56: 677–682.
- Wendland, J.R., Hampe, M., Newman, T.K., Syagailo, Y., Meyer, J., Schempp, W., Timme, A., Suomi, S.J. and Lesch, K.P. (2005) Structural variation of the monoamine oxidase A gene promoter repeat polymorphism in non human primates. *Genes Brain Behav.*, 5: 40–45.
- Wickens, M., Bernstein, D.S., Kimble, J. and Parker, R. (2002) A PUF family portrait: 3'UTR regulation as a way of life. *Trends Genet.*, 18: 150–157.
- Wilhelm, K., Mitchell, P.B., Niven, H., Finch, A., Wedgwood, L., Scimone, A., Blair, I.P., Parker, G. and Schofield, P.R. (2006) Life events, first depression onset and the serotonin transporter gene. *Br. J. Psychiatry*, 188: 210–215.
- Willeit, M., Stastny, J., Pirker, W., Praschak-Rieder, N., Neumeister, A., Asenbaum, S., Tauscher, J., Fuchs, K., Sieghart, W., Hornik, K., Aschauer, H.N., Brucke, T. and Kasper, S. (2001) No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biol. Psychiatry*, 50: 8–12.
- Williams, H.J., Owen, M.J. and O'Donovan, M.C. (2007) Is COMPT a susceptibility gene for schizophrenia? *Schizophr. Bull.*, 33: 635–641.
- Williams, J., Spurlock, G., Holmans, P., Mant, R., Murphy, K., Jones, L., Cardno, A., Asherson, P., Blackwood, D., Muir, W., Meszaros, K., Aschauer, H., Mallet, J., Laurent, C., Pekkarinen, P., Seppala, J., Stefanis, C.N., Papadimitriou, G.N., Macciardi, F., Verga, M., Pato, C., Azevedo, H., Crocq, M.A., Gurling, H., Owen, M.J., et al. (1998) A meta-analysis and transmission disequilibrium study of association between the dopamine D₃ receptor gene and schizophrenia. *Mol. Psychiatry*, 3: 141–149.

- Williams, J., Spurlock, G., McGuffin, P., Mallet, J., Nothen, M.M., Gill, M., Aschauer, H., Nylander, P.O., Macciardi, F. and Owen, M.J. (1996) Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptamine type 2a-receptor gene. *Lancet*, 347: 1294–1296.
- Willis-Owen, S.A.G., Fullerton, J., Surtees, P.G., Wainwright, N.W.J., Miller, S. and Flint, J. (2005) The Val66Met coding variant of the brain derived neurotrophic factor (BDNF) does not contribute toward variation in the personality trait neuroticism. *Biol. Psychiatry*, 58: 738–742.
- Wong, A.H.C., Buckle, C.E. and Van Tol, H.H.M. (2000) Polymorphisms in dopamine receptors: what do they tell us? *Eur. J. Pharmacol.*, 410: 183–203.
- Xing, Q.-H., Wu, S.-N., Lin, Z.-G., Li, H.-F., Yang, J.-D., Feng, G.-Y., Wang, M.-T., Yang, W.-W. and He, L. (2003) Association analysis of polymorphisms in the upstream region of the human dopamine D₄ receptor gene in schizophrenia. *Schizophr. Res.*, 65: 9–14.
- Yang, B., Chan, R.C.K., Jing, J., Li, T., Sham, P. and Chen, R.Y.L. (2007) A meta-analysis of association studies between the 10-repeat allele of a VNTR polymorphism in the 3'UTR of dopamine transporter gene and attention deficit hyperactivity disorder. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 144B: 541–550.
- Young, S.E., Smolen, A., Hewitt, J.K., Haberstick, J.K., Brett, C., et al. (2006) Interaction between MAOA genotype and maltreatment in the risk for conduct disorder: failure to confirm in adolescent patients. *Am. J. Psychiatry*, 163: 951–953.
- Zarrin, A.A., Malkin, L., Fong, I., Luk, K.D., Ghose, A. and Berinstein, N.L. (1999) Comparison of CNV, RSV, SV40 viral and Vlambda1 cellular promoters in B and T lymphoid and non-lymphoid cell lines. *Biochim. Biophys. Acta*, 1446: 135–139.
- Zhu, G., Lipsky, R.H., Xu, K., Ali, S., Hyde, T., Kleinman, J., Akhtar, L.A., Mash, D.C. and Goldman, D. (2004) Differential expression of human COMT alleles in brain and lymphoblasts detected by RT-coupled 5'nuclease assay. *Psychopharmacology (Berl.)*, 177: 178–184.
- Zhu, Q.-S., Grimsby, J., Chen, K. and Shih, J.C. (1992) Promoter organisation and activity of human monoamine oxidase (MAO) A and B genes. *J. Neurosci.*, 12: 4437–4446.
- Zhu, Q.-S. and Shih, J.C. (1997) An extensive repeat structure down-regulates human monoamine oxidase A promoter activity independent of an initiator-like sequence. *J. Neurochem.*, 69: 1368–1373.

CHAPTER 5

Distribution of 5-HT and DA receptors in primate prefrontal cortex: implications for pathophysiology and treatment

Julián de Almeida¹, José M. Palacios³ and Guadalupe Mengod^{1,2,*}

¹*Departament de Neuroquímica i Neurofarmacologia, Institut d'Investigacions Biomèdiques de Barcelona, CSIC-IDIBAPS, Barcelona, Spain*

²*Centro de Investigación Biomédica en Red Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain*

³*Universitat de Barcelona, Barcelona, Spain*

Abstract: The prefrontal cortex (PFC) has attracted a great research interest because of its involvement in the control of executive functions in both health and disease, and particularly in cognitive functions such as working memory. In schizophrenia, alterations in the PFC are documented at many different levels: molecular, cellular and functional. Furthermore, deficits in cognitive abilities are considered a core feature of schizophrenia and remain a major unmet medical need with respect to this disorder. In order to understand the sites of action of currently used drugs, as well as of the new experimental treatments being developed and acting in this brain region, it is important to have a detailed knowledge of the corresponding chemical neuroanatomy. Here we review current knowledge regarding the cellular localization of 5-HT_{1A}, 5-HT_{2A} and dopamine D₁, D₅, and D₂, D₄ receptors in primate PFC and their possible functions in the neuronal circuits of the PFC.

Keywords: serotonin receptors; dopamine receptors; GABAergic interneurons; glutamatergic cells

Prefrontal cortex: location, connectivity and cytoarchitecture

In mammals, the prefrontal cortex (PFC) is located in the anterior part of the frontal lobe, anterior to the motor and premotor cortical areas. It is traditionally divided into three major regions: orbitofrontal and ventromedial areas, dorsolateral prefrontal cortex (DLPFC), and the anterior and ventral cingulate cortex. The PFC comprises areas

8–13, 24, 25, 32 and 44–47 according to Brodmann (Brodmann and Garey, 2006). The dorsal PFC refers to Brodmann's area (BA) 8–10; the DLPFC to BA 46 and ventral to BA 9; the lateral PFC to BA 44, 45 and 47; the orbitofrontal PFC to areas BA 11 and 12; the anterior cingulate region to BA 24 and 25; and the cortex of the medial surface to BA 32 (Fallon et al., 2003). The PFC is described as a six-layered structure, as other neocortical regions, and it is distinguished by a granular layer IV (Fig. 1).

In primates, the PFC orchestrates thoughts and actions, as well as the planning of complex cognitive and affective functions, and it is therefore implicated in mood disorders (Ebert and

*Corresponding author. Tel.: 0034933638323;
Fax: 0034933638301

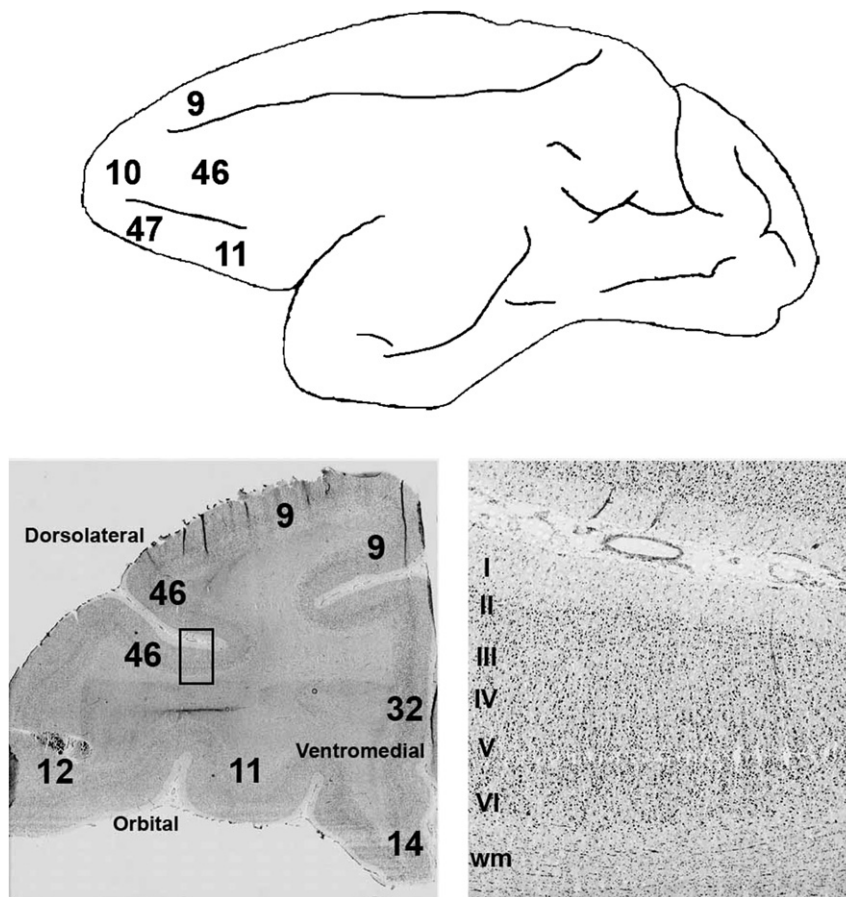


Fig. 1. The prefrontal cortex is a group of cortical areas located in the anterior part of the frontal lobe (upper panel) of the monkey brain. A coronal section of monkey PFC stained with cresyl violet shows the main divisions: orbital, ventromedial and dorsolateral, as well as some of the Brodmann areas. The small rectangle of area 46 is shown in the right at higher magnification, where the six laminae are numbered from the pial surface to the white matter, based on the density and the size of the cresyl violet-stained cells.

[Ebmeier, 1996](#)). The orbital and medial regions are involved in the control of emotional behaviour, whereas the lateral regions, which are highly developed in humans, provide cognitive support to the temporal organization of behaviour, speech and reasoning and the execution of complex behaviours that require working memory, as already mentioned; for reviews, see [Goldman-Rakic et al. \(1984\)](#), [Fuster \(1997\)](#), [Cavada et al. \(2000\)](#) and [Elston \(2003\)](#).

The PFC receives projections from the amygdala ([Barbas and de Olmos, 1990](#)), the ventral striatum ([Kunishio and Haber, 1994](#)) and thalamus ([Barbas et al., 1991](#)). Specifically, it receives

dopaminergic efferents from the ventral tegmental area (VTA) ([Lindvall et al., 1978](#); [Berger et al., 1988](#); [Conde et al., 1995](#)) and serotonergic innervation from the median and dorsal raphe nuclei ([Berger et al., 1988](#); [Smiley and Goldman-Rakic, 1996](#)), while it sends glutamatergic projections to both the VTA and the nucleus accumbens ([Sesack and Pickel, 1992](#); [Taber et al., 1995](#)), as well as to the raphe nuclei ([Sesack et al., 1989](#)). The PFC is connected with other association cortices but not with primary sensory or motor cortices. There is an important internal network of connectivity between the different divisions of the PFC; indeed, each of the three major prefrontal

regions (orbital, medial and lateral) is connected with itself and with the other two. Some of the cortico-cortical connectivity of the PFC is inter-hemispheric and the majority is organized in a reciprocal and topographical manner (Cavada and Goldman-Rakic, 1989a, b). These cortico-cortical connections originate and terminate in upper cortical layers II and III (Andersen et al., 1985).

As in other cortical regions, there are two main cell types in the PFC. One is the pyramidal and spiny stellate cells characterized by the excitatory asymmetric synapses which they form (glutamatergic). The majority of cortical neurons are pyramidal output neurons, found mainly in layers II–VI. The others are smooth or sparsely spiny neurons, which are interneurons that form inhibitory symmetric synapses (GABAergic) and are found in layers I–VI. In the cerebral cortex, several different subclasses of gamma aminobutyric acid (GABA)-containing neurons can be distinguished by their content of calcium-binding proteins and electrophysiological characteristics: parvalbumin (PV) cells with physiological properties of fast-spiking interneurons and the morphology of chandelier and large basket cells; and calbindin (CB) cells with physiological properties of non-fast-spiking interneurons, the majority of which show the morphology of double-bouquet cells (Conde et al., 1994; DeFelipe, 1997; Zaitsev et al., 2005).

Specific populations of PFC pyramidal neurons have been proposed to be responsible for maintaining ‘on-line’ the information required for working memory (Goldman-Rakic, 1995). Furthermore, inhibitory neurons in the PFC seem to play an important role in regulating the spatially tuned activity of pyramidal neurons during working memory. As mentioned above, there is also evidence that PFC dysfunction is a pathophysiological feature of schizophrenia, and a role has been demonstrated for GABAergic interneurons in the regulation of PFC function (Goldman-Rakic and Selemon, 1997; Lewis et al., 1999). Reduced expression for glutamic acid decarboxylase 67 (GAD67) (67 kDa isoform of glutamate decarboxylase), a synthesizing enzyme for GABA, has been found in the DLPFC of individuals with schizophrenia (Volk et al., 2000)

but only in a subset of these GABAergic neurons, the PV-positive neurons (Daviss and Lewis, 1995; Beasley et al., 2002; Hashimoto et al., 2003), which also had reduced but detectable levels of PV mRNA. Thus, the number of PV-expressing GABA neurons in the DLPFC of subjects with schizophrenia is unchanged, but they do have decreased expression of several genes, thus impairing cell function.

The major treatment for schizophrenia is anti-psychotic or neuroleptic drugs, all of which are characterized by their ability to interact with dopaminergic and serotonergic neurons. They are usually divided into two classes, called typical and atypical neuroleptics, differentiated by their profiles of unwanted side-effects. Additionally, the effects of these drugs on cognitive parameters in the disease are limited. In this regard, the need to develop new and more efficacious neuroleptics highlights the need to understand the cellular location of the sites of action of these drugs.

An important element in the chemical machinery of the brain is neurotransmitter receptors. Receptors for certain neurotransmitters, such as dopamine (DA) and serotonin, are relevant because they are the sites of action for drugs used in the treatment of psychiatric disorders. In this chapter, we review recent data on the cellular localization of specific subtypes of these receptors in the primate PFC with the goal of integrating this information on the molecular circuitry of this brain area and its relevance, thus laying the basis for the development of drugs to improve psychiatric disorders. The review covers recent data from our laboratory and others on the cellular localization of 5-HT_{1A}, 5-HT_{2A} and DA D1, D5, and D2, D4 in primate PFC. In these studies, we have used double in situ hybridization approaches, which enable the expression of receptors to be correlated with the expression of molecules that define the cellular phenotype. These results are analysed together with data on radioligand-binding autoradiography and immunohistochemistry using receptor antibodies in order to propose the localization of receptors and their possible functions in the neuronal circuits of the PFC. Table 1 summarizes current knowledge on the laminar and cellular localization of these receptors.

Table 1. Layer and cellular localization of dopamine and serotonin receptors in primate prefrontal cortex

	Receptor						
	D1	D5	D2	D3	D4	5-HT _{1A}	5-HT _{2A}
Layers							
Receptor autoradiography	I, II, V ^a , I–IIIa, V–VI ^b	nd	I, II, upper III ^c , V ^b	nd	nd	Superficial layers ^d	I, III–IV ^{e,f}
Receptor immunoreaction	II, III, V ^g	II, III, V ^g , IV–VI ^h	IV–V ⁱ	IV–V ⁱ	IV–V ⁱ	II–upper III ^j	II, III, V, VI ^k
Receptor mRNA	Deep layers ^l , V ^m , II, III, V, VI ⁿ	Deep layers ^{s,l} , V ^{m,n}	Superficial and deep layers ^{s,l} , V ^{m,n}	Deep layers ^{s,l} , V ^m	Superficial and deep layers ^l , V ^m , III–V ^o , II–VI ⁿ	Superficial layers ^{d,p,q}	III, V ^p , III–IV ^{e,f}
Cell type							
Pyramidal cells	+ ^g	+ ^{g,h}	+ ⁱ	nd	+ ^{i,o}	+ ^{p,q}	+ ^{f,k,p}
GABAergic cells	+ ^g	+ ^g	+ ⁱ	+ ⁱ	+ ^{i,o}	+ ^q	+ ^{f,k,p}
<i>Parvalbumin</i>	+ ^r	nd	nd	nd	nd	nd	+ ^f
<i>Calbindin</i>	nd	nd	nd	nd	nd	nd	+ ^{f,k}
<i>Calretinin</i>	+ / – ^r	nd	nd	nd	nd	nd	nd

^aCortés et al. (1989).^bLidow et al. (1991).^cCamps et al. (1989).^dMengod et al. (1996).^eLópez-Giménez et al. (2001).^fde Almeida and Mengod (2007).^gBergson et al. (1995b).^hKhan et al. (2000).ⁱKhan et al. (1998a).^jDeFelipe et al. (2001).^kJakab and Goldman-Rakic (1998).^lMeador-Woodruff et al. (1996).^mLidow et al. (1998).ⁿde Almeida and Mengod (in preparation).^oMrzljak et al. (1996).^pBurnet et al. (1995).^qde Almeida and Mengod (submitted).^rMuly et al. (1998).^sFaint expression.

5-HT receptors in the monkey PFC

The PFC receives serotonergic innervation from the medial and dorsal raphe nuclei (Steinbusch, 1981; Mamounas et al., 1991; Wilson and Molliver, 1991a, b). Serotonergic axons are present in all PFC layers, presenting a slight reduction in their density in layer III (Wilson and Molliver, 1991a), with beaded axons predominating in layer I and fine axons in layers II–VI. The serotonergic neurons synapse primarily on GABAergic interneurons (Smiley and Goldman-Rakic, 1996). The raphe nuclei receive reciprocal glutamatergic innervation from the PFC (Sesack et al., 1989).

The action of 5-hydroxytryptamine (5-HT) in these cells can be mediated by serotonin receptors. There are 7 subtypes of 5-HT receptors, which together with their different subtypes result in at least 14 different receptors, plus the many subtypes that result from alternative splicing (Hoyer et al., 2002). The involvement of serotonin in schizophrenia has been a subject of much interest (Breier, 1995) due to the particular pharmacological profile exhibited by atypical antipsychotic drugs such as clozapine, olanzapine and quetiapine, which are potent 5-HT_{2A} receptor antagonists and relatively weaker DA D2 receptor antagonists (Meltzer, 1999). Of the remaining 5-HT receptors with

which these drugs also interact, 5-HT_{1A} receptors have been postulated as additional good receptor candidates for their contribution to the antipsychotic action of these drugs. However, in contrast with 5-HT_{2A} receptors, where blockade is required for therapeutic activity, the stimulation of 5-HT_{1A} receptors appears to produce similar effects to the antagonism of 5-HT_{2A} receptors (Meltzer, 1999).

The involvement of serotonin in the pathophysiology of schizophrenia is supported by the clinical efficacy of drugs acting on 5-HT receptors. Additionally, in attempts to pinpoint specific brain regions and cells involved in the clinical activity of these drugs, a number of studies have examined the changes in post-mortem brain tissue from schizophrenic patients. Changes in 5-HT receptor densities have been described in different brain areas. For example, 5-HT_{1A} receptors have been found to have increased densities in the PFC of schizophrenic patients (Hashimoto et al., 1993; Sumiyoshi et al., 1996), whereas for 5-HT_{2A} receptors, the findings are less consistent since both increments (Joyce et al., 1993) and reduction (Dean et al., 1998) have been described in this brain area. The levels of mRNA coding for 5-HT_{2A} receptors are lower in PFC of schizophrenic patients (Burnet et al., 1996). They remained unchanged in patients treated with neuroleptics and dropped when patients had been free of neuroleptics for more than six months (Hernandez and Sokolov, 2000).

We will now review the recent data from our laboratory and others on the cellular localization of 5-HT_{1A} and 5-HT_{2A} receptors in PFC.

Cellular localization of 5-HT_{1A} in PFC

The 5-HT_{1A} receptor is negatively coupled to adenylate cyclase through the G protein G_{i/o}. This receptor can be considered as having the opposite functional effect to the 5-HT_{2A} receptor. Activation of the inhibitory 5-HT_{1A} autoreceptor on the raphe nucleus cells attenuates the firing of these neurons.

5-HT_{1A} receptors are found at high densities in the PFC of many species (Pazos and Palacios, 1985; Pazos et al., 1987a; Pompeiano et al., 1992;

Marazziti et al., 1994), preferentially in external cortical layers (Mengod et al., 1996). Immunohistochemical and in situ hybridization studies have revealed the presence of 5-HT_{1A} receptor protein and mRNA in external PFC layers in rat (Pompeiano et al., 1992; Kia et al., 1996; Santana et al., 2004; Abbas et al., 2007) and in monkey and human brain (Marazziti et al., 1994; Burnet et al., 1995; Mengod et al., 1996; Pasqualetti et al., 1996; DeFelipe et al., 2001; Cruz et al., 2004).

There is controversy as to the subcellular location of 5-HT_{1A} receptors. Riad et al. (2000) found a somatodendritic location of the receptor protein in rat brain. However, by using a different receptor antibody (Azmitia et al., 1996), 5-HT_{1A} receptor protein has also been localized in axons of pyramidal cells in rat, monkey and human (DeFelipe et al., 2001; Cruz et al., 2004). In the rat PFC, 60% of the glutamatergic cells express 5-HT_{1A} receptors and 25% of the GABAergic interneurons contain this receptor mRNA (Santana et al., 2004). Using dual in situ hybridization, we found that in monkey PFC, the percentage of glutamatergic cells containing 5-HT_{1A} receptor mRNA is higher than in rat, with 5-HT_{1A} receptor mRNA being expressed in about 80% of glutamatergic neurons in external layers II and upper III, and in around 50% in layer VI; they are also present in approximately 20% of GABAergic neurons (de Almeida and Mengod, submitted). An example of this distribution is shown in Fig. 2. 5-HT_{1A} receptor transcripts are abundantly expressed mainly in layers II and upper III, whereas they are less abundant in the cells of layer VI and show very low expression in layers III–V in both species. No 5-HT_{1A} receptor mRNA hybridization signal was seen in layer I. A notable co-localization of 5-HT_{1A} receptor mRNA with the glutamatergic marker vesicular glutamate transporter 1 (vGluT1) mRNA, as determined by double in situ hybridization histochemistry, can be appreciated in layers II–upper III in monkey PFC (Fig. 2b), which contrasts with the lower co-localization of 5-HT_{1A} receptor mRNA and GAD65/67 mRNA (Fig. 2c).

These results suggest that the binding sites localized by autoradiography correspond to those receptors visualized by both immunohistochemistry

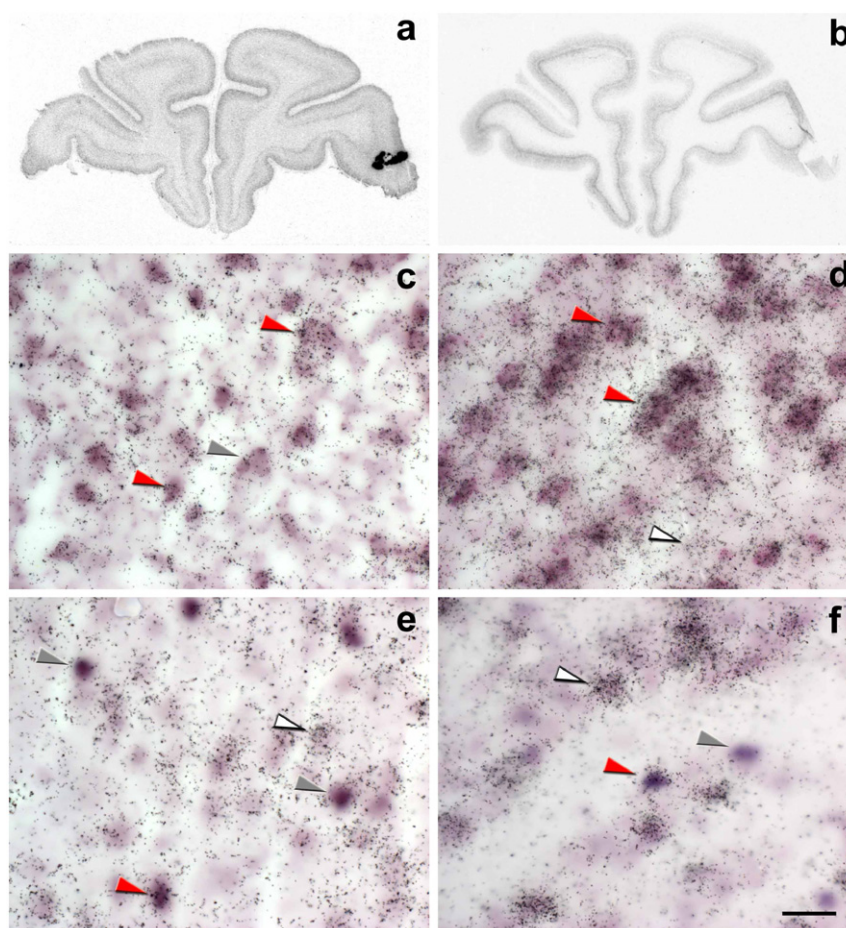


Fig. 2. Autoradiographical localization of mRNAs coding for 5-HT_{1A} (a) and 5-HT_{2A} (b) receptor mRNAs in monkey prefrontal cortex. Cellular localization of 5-HT_{1A} (c, e) and 5-HT_{2A} (d, f) receptor mRNAs in glutamatergic (c, d) and GABAergic (e, f) cell populations in monkey PFC. High-magnification bright-field microphotographs of emulsion-dipped sections of layers II–upper III of the dorsolateral prefrontal cortex, simultaneously showing the different mRNAs visualized by double in situ hybridization using ³³P-labelled oligonucleotides complementary to the mRNA coding for serotonin receptors 5-HT_{1A} and 5-HT_{2A} (clusters of dark silver grains), with DIG-labelled oligonucleotides (dark precipitate) for vGluT1 mRNA (glutamatergic cells), panels c and d, or for GAD65/67 mRNA (GABAergic cells), panels e and f. The white arrow head indicates a radioactively labelled cell, the grey arrow head points to DIG-labelled cells and the red arrow head to a double-labelled cell. Bars = 5.2 mm (a, b) and 30 μ m (c–f). (See Color Plate 5.2 in color plate section.)

and in situ hybridization, due to the well-known somatodendritic localization of these receptors. These data point to the pyramidal cells of layers II–III as being one of the main cells in PFC that expresses 5-HT_{1A} receptors. Since these cells are known to be involved in cortico-cortical projections, both contralateral and ipsilateral, it would seem that these receptors play a role in these connections. 5-HT_{1A} receptors are also found in

GABAergic cells, although in a lower number of cells, thus providing a second way of influencing the role of 5-HT in pyramidal and GABAergic cells.

Cellular localization of 5-HT_{2A} in PFC

The 5-HT_{2A} receptor activates phospholipase C by coupling to G proteins. As mentioned above, this

receptor can be considered as having the opposite functional effect to the 5-HT_{1A} receptor.

5-HT_{2A} receptors are found at high densities in the PFC of many species (Pazos et al., 1985, 1987b; Pompeiano et al., 1994; López-Giménez et al., 2001). In monkey PFC, layers I and III–IV presented the highest densities of 5-HT_{2A}-labelled receptors (López-Giménez et al., 2001). Immunohistochemical and in situ hybridization studies have revealed the presence of 5-HT_{2A} receptors in both rat (Pompeiano et al., 1994; Willins et al., 1997; Cornea-Hebert et al., 1999; Xu and Pandey, 2000; Martin-Ruiz et al., 2001) and monkey and human (Jakab and Goldman-Rakic, 1998, 2000; López-Giménez et al., 2001; de Almeida and Mengod, 2007) PFC. Layers III and IV showed the highest hybridization levels in monkey PFC (López-Giménez et al., 2001; de Almeida and Mengod, 2007), although Burnet et al. (1995) observed that 5-HT_{2A} mRNA was concentrated in two bands, probably corresponding to lamina III and V in the orbitofrontal cortex.

Whereas the pyramidal neuron is the major cortical cell type expressing 5-HT_{2A} receptors, some cortical GABAergic interneurons have also been found to express these receptors in the rat (Willins et al., 1997; Santana et al., 2004) and primate brain (Burnet et al., 1995; Jakab and Goldman-Rakic, 1998; de Almeida and Mengod, 2007). Several groups have shown that the PFC 5-HT_{2A} receptor protein is localized in the apical dendrites of pyramidal cells (Willins et al., 1997; Jakab and Goldman-Rakic, 1998). In PFC interneurons, 5-HT_{2A} receptor expression is found in CB-positive cells (Jakab and Goldman-Rakic, 1998). Figure 2d shows that 5-HT_{2A} receptor transcripts are abundantly expressed in the monkey PFC in a large number of cells that are distributed preferentially between layers III and V, whereas they are less abundant in the cells of layers II and VI and absent in layer I. It is worth mentioning that the largest amount of 5-HT_{2A} receptor mRNA is found in layer V. Glutamatergic neurons labelled by a dark precipitate are found in all layers, except layer I. PFC cells expressing both GABAergic cell markers, GAD65 and GAD67, are found scattered throughout the PFC, including layer I. The great majority of

glutamatergic cells in layers II–V expressed 5-HT_{2A} receptors in the nine prefrontal areas examined (86–100%), with a maximum (almost 100%) observed in layers III and V. In GABAergic cells in layers II–V, this percentage was lower (13–31%). This difference in the percentage of the two cellular populations was, however, much lower when layer VI was analysed. The proportion of glutamatergic cells expressing 5-HT_{2A} receptors decreased to 52–72%, whereas GABAergic interneurons expressing this receptor increased to 28–46%. This receptor is expressed in 45–69% of PV and in 61–87% of CB-positive cells (de Almeida and Mengod, 2007).

This cellular location, in pyramidal cells of layers III and V, suggests that these receptors are involved in contralateral cortico-cortical connections and subcortical connections.

Dopamine receptors

DA has been associated with functions such as motivation, affect, reward, movement and performance on cognitive tasks. Cognitive symptoms have been linked with DA dysregulation in several diseases, including schizophrenia (Knable and Weinberger, 1997).

The dopaminergic system arises from cells located in the midbrain. Anatomical studies in rodents demonstrate that afferents from the PFC innervate the VTA GABAergic cells which, in turn, project to the nucleus accumbens (Sesack et al., 1989; Hurley et al., 1991; Sesack and Pickel, 1992) and the VTA DA cells that project back to the PFC (Carr and Sesack, 2000) (mesocortical projections). Dopaminergic neurons synapse on at least two cellular populations: pyramidal excitatory neurons (glutamate) and non-pyramidal GABA interneurons (Goldman-Rakic et al., 1989; Cowan et al., 1994; Sesack et al., 1995).

The central actions of DA are mediated by DA receptors, which are classified into D1-like or D2-like receptors based on their pharmacological or functional profile (Kebabian and Calne, 1979; Missale et al., 1998; Vallone et al., 2000). Receptors belonging to the D1 family (D1 and D5) are positively linked to adenylyl cyclase,

whereas those belonging to the D2 family (D2, D3 and D4) are negatively coupled to adenylyl cyclase or to other transduction pathways.

The pharmacological differences between D1-like and D2-like receptors described almost 30 years ago (Kebabian and Calne, 1979) remain valid today. The number of purely selective ligands for each receptor is very limited (Alexander et al., 2004). Most of the work done on DA receptors has been centred on subcortical regions such as the striatum, where the density of these receptors is very high (Palacios et al., 1988; Camps et al., 1989; Cortés et al., 1989). Since it was first discovered in the 1970s that the effects of many antipsychotic drugs correlated with their affinities to D2 receptors (Creese et al., 1976), most of the research on the aetiology and treatment of schizophrenia has been centred on these receptors. Subsequently, and given the involvement of cortex and particularly the PFC in schizophrenia (Weinberger, 1988), the study of the expression of DA receptors in this brain area has become an important issue.

Cellular localization of D1-like receptors in the PFC

DA binds to D5 receptors with a 5–10-fold higher affinity than for D1 receptors (Sunahara et al., 1991). As there are no pharmacological tools capable of differentiating between D1 and D5 receptors, the pharmacological effects of D1 agonists are likely to be mediated by both D1 and D5 receptors. The only way to functionally dissect these two receptor subtypes is by knock out mice and antisense strategies (Sibley, 1999). The D1 family of DA receptors is very abundant in the neocortex (Cortés et al., 1989). Receptor autoradiography studies with D1-specific ligands have demonstrated that D1-like receptors are present in both human (Cortés et al., 1989) and monkey neocortex at high densities in superficial layers I–IIIa and in deep layers V–VI, as well as at lower densities in layers IIIb–IV (Lidow et al., 1991).

High hybridization levels for D1 and D5 receptor mRNAs have been described in the primate neocortex (Huntley et al., 1992; Meador-Woodruff et al., 1996). In human PFC, Meador-Woodruff et al. (1996) described the presence of

D1 mRNA receptors in the deeper layers, with faint labelling in more superficial layers. In situ hybridization studies to examine the expression of D1 and D5 receptor mRNAs in PFC showed their presence primarily in cell layer V in both human and non-human primates (Lidow et al., 1998). In monkey PFC, D1 mRNA can also be found in layer II and part of layer VI in addition to layer V, whereas D5 mRNA is found exclusively in layer V (de Almeida and Mengod, in preparation) (Fig. 3).

D1-like immunoreactivity is present in cortical interneurons of monkey, it being prevalent in PV-containing neurons and less common in calretinin-containing interneurons (Muly et al., 1998). However, by using another antibody, Paspalas and Goldman-Rakic (2005) were unable to detect any D1 immunoreaction in PV-positive cells in monkey PFC. In monkey PFC, D1-like immunoreactivity is found preferentially in the distal dendrites and spines of pyramidal cells, as well as on the dendrites and axon terminals of putative GABAergic interneurons (Smiley et al., 1994; Bergson et al., 1995b; Muly et al., 1998), whereas D5 receptors are located on perikarya and proximal dendrites of many pyramidal and some non-pyramidal neurons (Bergson et al., 1995a, b; Ciliax et al., 2000; Khan et al., 2000) in layers IV–VI. D1 immunoreactivity for the heteroreceptor is distinctly localized on perisynaptic and extrasynaptic membranes of excitatory-like varicosities (Paspalas and Goldman-Rakic, 2005).

Cellular localization of D2-like receptors in the PFC

The distribution of D2-like receptors in the monkey and human brain has been analysed by receptor radioligand autoradiography (Camps et al., 1989; Goldman-Rakic et al., 1989; Lidow et al., 1989; Mengod et al., 1992).

In human PFC, Meador-Woodruff et al. (1996) described the faint presence of D2 mRNA receptors in both superficial and deep layers of the PFC. The D4 mRNA receptor is more abundant and was found to be expressed in the deeper layers with faint labelling in more superficial layers, there being an apparent enrichment in the deep cortex. The same authors comment that

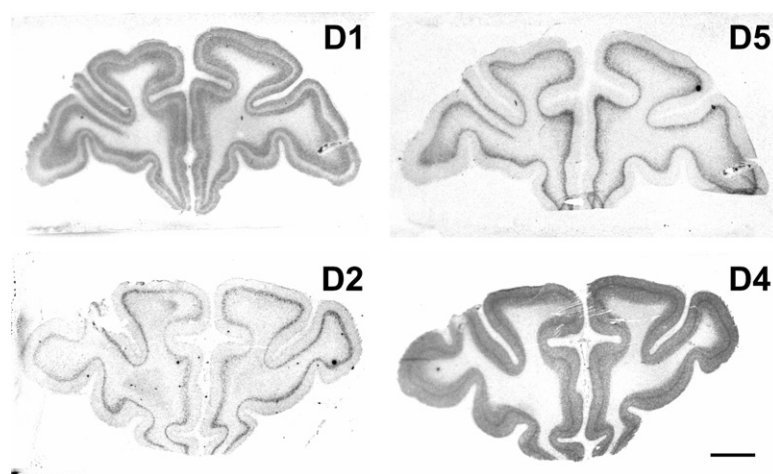


Fig. 3. Autoradiographical localization of mRNAs coding for D1, D5, D2 and D4 receptor mRNAs in monkey prefrontal cortex. D1 mRNA is found in layers II, III, V and VI. D5 and D2 mRNAs are found in layer V. D4 mRNA is present in layers II–VI, and is especially abundant in layer V. Bar = 5 mm.

the expression of D3 and D5 receptor mRNAs in PFC is particularly rare and they appear to be expressed in the deeper layers. In monkey PFC, the mRNA coding for D2, D3 and D4 receptor subtypes is present in all the cellular layers but is especially abundant in layer V (Lidow et al., 1998). Work in progress in our laboratory shows that in monkey PFC, D2 mRNA is found exclusively in layer V, whereas D4 mRNA can also be found in almost all layers (except in layer I) and at high levels in layer V (de Almeida and Mengod, in preparation) (Fig. 3).

D4 immunoreactivity has been detected in both pyramidal and non-pyramidal cells of human cortical areas including PFC (Khan et al., 1998a), whereas D2 and D3 immunostaining was found to be mostly associated with non-pyramidal neurons (Khan et al., 1998a, b). About 60% of cortical interneurons showed labelling in soma and dendritic shafts (Khan et al., 2001), while a subset (40%) of these showed immunolabelling in astrocytic processes enwrapping the cell bodies. These authors estimate that approximately 35% of the total D2 receptor-binding activity in the cortex may be associated with astrocytes. In primate brain, the D4 receptor antibody labelled GABAergic neurons in cerebral cortex, some of which were PV (Mrzljak et al., 1996). D2 receptor IR is

localized in distal dendritic and axonal processes (Negyessy and Goldman-Rakic, 2005).

Interaction of 5-HT, dopamine and their receptors: involvement in schizophrenia

The successful therapeutic application of antipsychotics, such as clozapine, olanzapine, seroquel and sertindole, has focused much research attention on understanding the interaction between the serotonergic and dopaminergic systems. As one of the characteristics of these compounds is their ability to block both DA and serotonin receptors, the initial focus has been on DA D2 and 5-HT₂ receptors (Meltzer et al., 1989). More recently, the efficacy and tolerability of the 'atypical' antipsychotic drugs is attributed in part to their interaction with specific serotonin receptors such as 5-HT_{1A} and 5-HT_{2A}.

At the neuroanatomical level, the interaction between 5-HT and DA mechanisms has been analysed in different pathways. Serotonin from the dorsal raphe inhibits the firing of dopaminergic neurons in the substantia nigra and antagonizes DA-mediated behaviours. This action is modulated by 5-HT₂ receptors located in the dopaminergic neurons. The raphe also projects to the

striatum and the release of 5-HT is also associated with an inhibition of striatal neuronal firing. In addition, serotonergic influence on striatal cholinergic and GABAergic systems is also well documented (Kapur and Remington, 1996; Werkman et al., 2006; Alex and Pehek, 2007). Again, the role of 5-HT₂ receptors in mediating the inhibitory effects of serotonin on dopaminergic activity is well documented. Additionally, 5-HT_{1A} agonists have also been found to reverse catalepsy in rodents and extrapyramidal symptoms in primate models by acting on the firing of serotonergic neurons, suggesting that the combination of 5-HT_{1A} agonist and D2 antagonist could result in an improved antipsychotic profile (Meltzer et al., 2003; Newman-Tancredi et al., 2005). While most studies have focused on dopaminergic transmission, understanding the serotonin–dopaminergic interactions involved in the mechanism of action of neuroleptics will require a different viewpoint as regards the situation in the cortex.

The PFC is the most appropriate region in which to look for interactions between DA and serotonin through their cortical receptors, located in either pyramidal cells or GABAergic interneurons. For example, it has been shown that the increase in PFC DA release produced by atypical antipsychotics such as clozapine, olanzapine and ziprasidone, but not by haloperidol, seems to involve 5-HT_{1A} receptor activation (Ichikawa et al., 2001; Diaz-Mataix et al., 2005). 5-HT_{1A} receptor agonists increase DA release in PFC, suggesting that this is a potential basis for the action of at least some of the atypical antipsychotics (Rollema et al., 1997; Sakaue et al., 2000).

The role of the cortical glutamatergic pyramidal neuron is to integrate thousands of afferent inputs from GABAergic interneurons, as well as from serotonergic and dopaminergic fibres, and also to control movement and affect through its efferent projection (Goldman-Rakic et al., 2000). DA axons represent a significant source of afferentation of PFC in primates (Williams and Goldman-Rakic, 1993, 1998), with area 9 being the one having the greatest density of dopaminergic fibres. Dopaminergic innervation in PFC reveals, in general, a bilaminar pattern of distribution that is especially dense in layer I and less so in layer II,

with intermediate density being observed in layers V and VI (Lewis, 1992). The PFC in primates also receives serotonergic innervation from the medial and dorsal raphe nuclei (Wilson and Molliver, 1991a, b). 5-HT axon fibres are found in a moderate density in all layers in monkey DLPFC (Wilson and Molliver, 1991a). DeFelipe et al. (2001) found a distinct density of 5-HT fibres in supragranular layers (I–III) and a much lower one in infragranular layers. The serotonergic neurons from the raphe nuclei synapse primarily on GABAergic interneurons (Smiley and Goldman-Rakic, 1996). The raphe nuclei receive reciprocal glutamatergic innervation from the pyramidal cells of PFC (Sesack et al., 1989).

The laminar distribution of cell bodies expressing 5-HT_{1A} and 5-HT_{2A} receptors shows a ‘complementary’ pattern, since 5-HT_{1A} receptors are mainly found in superficial layer II and part of layer III and at lower levels in deep layer VI, whereas 5-HT_{2A} receptors are located in intermediate layers III–V. This distribution could be related in part with the presence of different types of 5-HT axons (Wilson and Molliver, 1991a; Raghanti et al., 2008), as discussed above. These two 5-HT receptors are present in most PFC pyramidal neurons, where they could probably be co-expressed in some cells, while only a low proportion of cortical GABAergic cells express either receptor. Pyramidal 5-HT_{2A} receptors can modulate excitatory glutamate inputs (Puig et al., 2003), whereas 5-HT_{1A} receptors could act by reducing glutamate release in the thalamus.

All DA receptors are found to be expressed in PFC layer V (Lidow et al., 1998); additionally, D1 and D4 are found in other PFC layers in monkey (de Almeida and Mengod, in preparation), except in layer I. The observation that schizophrenia is associated with an altered DA innervation of PFC area 9, which is lamina- and neurotransmitter specific (Akil et al., 1999), indicates the importance of understanding the laminar (and subsequently, cell type) distribution of both 5-HT and DA receptors.

In non-human primates, it has been established that members of the D1-like receptor subfamily modulate excitatory transmission in prefrontal microcircuits, generating stimulus-independent

activity that is essential for working memory (Williams and Goldman-Rakic, 1995); furthermore, although the cellular basis is unknown, it is thought to involve D1-like receptor modulation of pyramidal neuron excitability in a layer- and input-specific manner (Seamans and Yang, 2004).

The co-expression of DA and serotonin receptors in the same PFC cells has not yet been analysed in any species. However, the only cortical layer where this could occur is layer V (Table 1), where most of these receptors, with the exception of 5-HT_{1A}, are present. Greater knowledge about this aspect could therefore help in understanding the array of electrophysiological data concerning the actions of antipsychotic drugs in PFC.

Conclusions

In conclusion, we have reviewed the current neuroanatomical data regarding a role for DA and serotonin in the functions of primate PFC through different receptor subtypes, namely 5-HT_{1A}, 5-HT_{2A}, D1, D2, D4 and D5, which are expressed in this brain area. The specific cellular populations expressing these receptors have been identified using *in situ* hybridization techniques.

One of the major findings of these studies is the non-overlapping localization of 5-HT_{1A} and 5-HT_{2A} receptors in the primate PFC with regards to their laminar localization and the involvement of cortical circuitry and projections. This suggests complementary rather than redundant roles for these receptors in regulating functions of the PFC, in addition to their excitatory or inhibitory nature.

Abbreviations

BA	Brodmann's area
CB	calbindin
DA	dopamine
DLPFC	dorsolateral prefrontal cortex
GABA	gamma aminobutyric acid
GAD	glutamic acid decarboxylase
5-HT	5-hydroxytryptamine or serotonin
PFC	prefrontal cortex
PV	parvalbumin

vGluT1	vesicular glutamate transporter 1
VTA	ventral tegmental area

Acknowledgements

J. de Almeida is the recipient of a fellowship from the Spanish Ministry of Education. This research was funded by the Fundació La Marató TV3 (#01/3930). Support from the Generalitat de Catalunya (Grup de Recerca de Qualitat 2005-SGR0758) is also acknowledged. We thank Robin Rycroft for English corrections.

References

- Abbas, S.Y., Nogueira, M.I. and Azmitia, E.C. (2007) Antagonist-induced increase in 5-HT_{1A}-receptor expression in adult rat hippocampus and cortex. *Synapse*, 61(7): 531–539.
- Akil, M., Pierri, J.N., Whitehead, R.E., Edgar, C.L., Mohila, C., Sampson, A.R. and Lewis, D.A. (1999) Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am. J. Psychiatry*, 156(10): 1580–1589.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113(2): 296–320.
- Alexander, S.P.H., Mathie, A. and Peters, J.A. (2004) Guide to receptors and channels, 1st edition. *Br. J. Pharmacol.*, 141: S1–S126.
- Andersen, R.A., Asanuma, C. and Cowan, W.M. (1985) Callosal and prefrontal associational projecting cell populations in area 7A of the macaque monkey: a study using retrogradely transported fluorescent dyes. *J. Comp. Neurol.*, 232(4): 443–455.
- Azmitia, E.C., Gannon, P.J., Kheck, N.M. and Whitaker-Azmitia, P.M. (1996) Cellular localization of the 5-HT_{1A} receptor in primate brain neurons and glial cells. *Neuropsychopharmacology*, 14(1): 35–46.
- Barbas, H. and de Olmos, J. (1990) Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *J. Comp. Neurol.*, 300(4): 549–571.
- Barbas, H., Henion, T.H. and Dermon, C.R. (1991) Diverse thalamic projections to the prefrontal cortex in the rhesus monkey. *J. Comp. Neurol.*, 313(1): 65–94.
- Beasley, C.L., Zhang, Z.J., Patten, I. and Reynolds, G.P. (2002) Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biol. Psychiatry*, 52(7): 708–715.
- Berger, B., Trotter, S., Verney, C., Gaspar, P. and Alvarez, C. (1988) Regional and laminar distribution of the dopamine and serotonin innervation in the macaque cerebral cortex: a radioautographic study. *J. Comp. Neurol.*, 273(1): 99–119.

- Bergson, C., Mrzljak, L., Lidow, M.S., Goldman-Rakic, P.S. and Levenson, R. (1995a) Characterization of subtype-specific antibodies to the human D5 dopamine receptor: studies in primate brain and transfected mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.*, 92(8): 3468–3472.
- Bergson, C., Mrzljak, L., Smiley, J.F., Pappy, M., Levenson, R. and Goldman-Rakic, P.S. (1995b) Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J. Neurosci.*, 15(12): 7821–7836.
- Breier, A. (1995) Serotonin, schizophrenia and antipsychotic drug action. *Schizophr. Res.*, 14(3): 187–202.
- Brodmann, K. and Garey, L.J. (2006) Brodmann's Localisation in the Cerebral Cortex. *The Principles of Comparative Localisation in the Cerebral Cortex based on Cytoarchitectonics* (3rd edn.). Springer, New York.
- Burnet, P.W., Eastwood, S.L. and Harrison, P.J. (1996) 5-HT1A and 5-HT2A receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology*, 15(5): 442–455.
- Burnet, P.W., Eastwood, S.L., Lacey, K. and Harrison, P.J. (1995) The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. *Brain Res.*, 676(1): 157–168.
- Camps, M., Cortés, R., Gueye, B., Probst, A. and Palacios, J.M. (1989) Dopamine receptors in human brain: autoradiographic distribution of D2 sites. *Neuroscience*, 28(2): 275–290.
- Carr, D.B. and Sesack, S.R. (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J. Neurosci.*, 20(10): 3864–3873.
- Cavada, C., Company, T., Tejedor, J., Cruz-Rizzolo, R.J. and Reinoso-Suarez, F. (2000) The anatomical connections of the macaque monkey orbitofrontal cortex. A review. *Cereb. Cortex*, 10(3): 220–242.
- Cavada, C. and Goldman-Rakic, P.S. (1989a) Posterior parietal cortex in rhesus monkey: I. Parcellation of areas based on distinctive limbic and sensory corticocortical connections. *J. Comp. Neurol.*, 287(4): 393–421.
- Cavada, C. and Goldman-Rakic, P.S. (1989b) Posterior parietal cortex in rhesus monkey: II. Evidence for segregated corticocortical networks linking sensory and limbic areas with the frontal lobe. *J. Comp. Neurol.*, 287(4): 422–445.
- Ciliax, B.J., Nash, N., Heilman, C., Sunahara, R., Hartney, A., Tiberi, M., Rye, D.B., Caron, M.G., Niznik, H.B. and Levey, A.I. (2000) Dopamine D(5) receptor immunolocalization in rat and monkey brain. *Synapse*, 37(2): 125–145.
- Conde, F., Lund, J.S., Jacobowitz, D.M., Baimbridge, K.G. and Lewis, D.A. (1994) Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefrontal cortex: distribution and morphology. *J. Comp. Neurol.*, 341(1): 95–116.
- Conde, F., Maire-Lepoivre, E., Audinat, E. and Crepel, F. (1995) Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J. Comp. Neurol.*, 352(4): 567–593.
- Cornea-Hebert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, 409(2): 187–209.
- Cortés, R., Gueye, B., Pazos, A., Probst, A. and Palacios, J.M. (1989) Dopamine receptors in human brain: autoradiographic distribution of D1 sites. *Neuroscience*, 28(2): 263–273.
- Cowan, R.L., Sesack, S.R., Van Bockstaele, E.J., Branchereau, P., Chain, J. and Pickel, V.M. (1994) Analysis of synaptic inputs and targets of physiologically characterized neurons in rat frontal cortex: combined in vivo intracellular recording and immunolabeling. *Synapse*, 17(2): 101–114.
- Creese, I., Burt, D.R. and Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192(4238): 481–483.
- Cruz, D.A., Eggan, S.M., Azmitia, E.C. and Lewis, D.A. (2004) Serotonin1A receptors at the axon initial segment of prefrontal pyramidal neurons in schizophrenia. *Am. J. Psychiatry*, 161(4): 739–742.
- Daviss, S.R. and Lewis, D.A. (1995) Local circuit neurons of the prefrontal cortex in schizophrenia: selective increase in the density of calbindin-immunoreactive neurons. *Psychiatry Res.*, 59(1–2): 81–96.
- de Almeida, J. and Mengod, G. (2007) Quantitative analysis of glutamatergic and GABAergic neurons expressing 5-HT(2A) receptors in human and monkey prefrontal cortex. *J. Neurochem.*, 103(2): 475–486.
- Dean, B., Hayes, W., Hill, C. and Copolov, D. (1998) Decreased serotonin2A receptors in Brodmann's area 9 from schizophrenic subjects. A pathological or pharmacological phenomenon? *Mol. Chem. Neuropathol.*, 34(2–3): 133–145.
- DeFelipe, J. (1997) Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28 K, parvalbumin and calretinin in the neocortex. *J. Chem. Neuroanat.*, 14(1): 1–19.
- DeFelipe, J., Arellano, J.I., Gomez, A., Azmitia, E.C. and Munoz, A. (2001) Pyramidal cell axons show a local specialization for GABA and 5-HT inputs in monkey and human cerebral cortex. *J. Comp. Neurol.*, 433(1): 148–155.
- Diaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25(47): 10831–10843.
- Ebert, D. and Ebmeier, K.P. (1996) The role of the cingulate gyrus in depression: from functional anatomy to neurochemistry. *Biol. Psychiatry*, 39(12): 1044–1050.
- Elston, G.N. (2003) Cortex, cognition and the cell: new insights into the pyramidal neuron and prefrontal function. *Cereb. Cortex*, 13(11): 1124–1138.
- Fallon, J.H., Opole, I.O. and Potkin, S.G. (2003) The neuroanatomy of schizophrenia: circuitry and neurotransmitter systems. *Clin. Neurosci. Res.*, 3: 77–107.
- Fuster, J.M. (1997) In: Placito M. and Bialer M. (Eds.), *The Prefrontal Cortex. Anatomy, Physiology, and*

- Neuropsychology of the Frontal Lobe (3rd edn.). Lippincott-Raven, Philadelphia, PA.
- Goldman-Rakic, P.S. (1995) Cellular basis of working memory. *Neuron*, 14(3): 477–485.
- Goldman-Rakic, P.S., Leranth, C., Williams, S.M., Mons, N. and Geffard, M. (1989) Dopamine synaptic complex with pyramidal neurons in primate cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 86(22): 9015–9019.
- Goldman-Rakic, P.S., Muly, E.C., III and Williams, G.V. (2000) D(1) receptors in prefrontal cells and circuits. *Brain Res. Brain Res. Rev.*, 31(2–3): 295–301.
- Goldman-Rakic, P.S. and Selemon, L.D. (1997) Functional and anatomical aspects of prefrontal pathology in schizophrenia. *Schizophr. Bull.*, 23(3): 437–458.
- Goldman-Rakic, P.S., Selemon, L.D. and Schwartz, M.L. (1984) Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience*, 12(3): 719–743.
- Hashimoto, T., Kitamura, N., Kajimoto, Y., Shirai, Y., Shirakawa, O., Mita, T., Nishino, N. and Tanaka, C. (1993) Differential changes in serotonin 5-HT_{1A} and 5-HT₂ receptor binding in patients with chronic schizophrenia. *Psychopharmacology*, 112(Suppl. 1): S35–S39.
- Hashimoto, T., Volk, D.W., Eggan, S.M., Mirnics, K., Pierri, J.N., Sun, Z., Sampson, A.R. and Lewis, D.A. (2003) Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J. Neurosci.*, 23(15): 6315–6326.
- Hernandez, I. and Sokolov, B.P. (2000) Abnormalities in 5-HT_{2A} receptor mRNA expression in frontal cortex of chronic elderly schizophrenics with varying histories of neuroleptic treatment. *J. Neurosci. Res.*, 59(2): 218–225.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Huntley, G.W., Morrison, J.H., Prikhozhan, A. and Sealfon, S.C. (1992) Localization of multiple dopamine receptor subtype mRNAs in human and monkey motor cortex and striatum. *Brain Res. Mol. Brain Res.*, 15(3–4): 181–188.
- Hurley, K.M., Herbert, H., Moga, M.M. and Saper, C.B. (1991) Efferent projections of the infralimbic cortex of the rat. *J. Comp. Neurol.*, 308(2): 249–276.
- Ichikawa, J., Ishii, H., Bonaccorso, S., Fowler, W.L., O’Laughlin, I.A. and Meltzer, H.Y. (2001) 5-HT_{2A} and D(2) receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.*, 76(5): 1521–1531.
- Jakab, R.L. and Goldman-Rakic, P.S. (1998) 5-Hydroxytryptamine_{2A} serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc. Natl. Acad. Sci. U.S.A.*, 95(2): 735–740.
- Jakab, R.L. and Goldman-Rakic, P.S. (2000) Segregation of serotonin 5-HT_{2A} and 5-HT₃ receptors in inhibitory circuits of the primate cerebral cortex. *J. Comp. Neurol.*, 417(3): 337–348.
- Joyce, J.N., Shane, A., Lexow, N., Winokur, A., Casanova, M.F. and Kleinman, J.E. (1993) Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. *Neuropsychopharmacology*, 8: 315–336.
- Kapur, S. and Remington, G. (1996) Serotonin–dopamine interaction and its relevance to schizophrenia. *Am. J. Psychiatry*, 153(4): 466–476.
- Kebabian, J.W. and Calne, D.B. (1979) Multiple receptors for dopamine. *Nature*, 277(5692): 93–96.
- Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A. and de la Calle, A. (1998a) Differential regional and cellular distribution of dopamine D₂-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. *J. Comp. Neurol.*, 402(3): 353–371.
- Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A. and de la Calle, A. (2000) Dopamine D₅ receptors of rat and human brain. *Neuroscience*, 100(4): 689–699.
- Khan, Z.U., Koulen, P., Rubinstein, M., Grandy, D.K. and Goldman-Rakic, P.S. (2001) An astroglia-linked dopamine D₂-receptor action in prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 98(4): 1964–1969.
- Khan, Z.U., Mrzljak, L., Gutierrez, A., de la Calle, A. and Goldman-Rakic, P.S. (1998b) Prominence of the dopamine D₂ short isoform in dopaminergic pathways. *Proc. Natl. Acad. Sci. U.S.A.*, 95(13): 7731–7736.
- Kia, H.K., Miquel, M.C., Brisorgueil, M.J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M. and Vergé, D. (1996) Immunocytochemical localization of serotonin(1A) receptors in the rat central nervous system. *J. Comp. Neurol.*, 365: 289–305.
- Knable, M.B. and Weinberger, D.R. (1997) Dopamine, the prefrontal cortex and schizophrenia. *J. Psychopharmacol.*, 11(2): 123–131.
- Kunishio, K. and Haber, S.N. (1994) Primate cingulostriatal projection: limbic striatal versus sensorimotor striatal input. *J. Comp. Neurol.*, 350(3): 337–356.
- Lewis, D.A. (1992) The catecholaminergic innervation of primate prefrontal cortex. *J. Neural Transm. Suppl.*, 36: 179–200.
- Lewis, D.A., Pierri, J.N., Volk, D.W., Melchitzky, D.S. and Woo, T.U. (1999) Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biol. Psychiatry*, 46(5): 616–626.
- Lidow, M.S., Goldman-Rakic, P.S., Gallagher, D.W. and Rakic, P. (1991) Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [³H]raclopride, [³H]spiperone and [³H]SCH23390. *Neuroscience*, 40(3): 657–671.
- Lidow, M.S., Goldman-Rakic, P.S., Rakic, P. and Innis, R.B. (1989) Dopamine D₂ receptors in the cerebral cortex: distribution and pharmacological characterization with [³H]raclopride. *Proc. Natl. Acad. Sci. U.S.A.*, 86(16): 6412–6416.

- Lidow, M.S., Wang, F., Cao, Y. and Goldman-Rakic, P.S. (1998) Layer V neurons bear the majority of mRNAs encoding the five distinct dopamine receptor subtypes in the primate prefrontal cortex. *Synapse*, 28(1): 10–20.
- Lindvall, O., Bjorklund, A. and Divac, I. (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. *Brain Res.*, 142(1): 1–24.
- López-Giménez, J.F., Vilaró, M.T., Palacios, J.M. and Mengod, G. (2001) Mapping of 5-HT_{2A} receptors and their mRNA in monkey brain: [3H]MDL100,907 autoradiography and in situ hybridization studies. *J. Comp. Neurol.*, 429(4): 571–589.
- Mamounas, L.A., Mullen, C.A., O'Hearn, E. and Molliver, M.E. (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J. Comp. Neurol.*, 314(3): 558–586.
- Marazziti, D., Marracci, S., Palego, L., Rotondo, A., Mazzanti, C., Nardi, I., Ladinsky, H., Giraldo, E., Borsini, F. and Cassano, G.B. (1994) Localization and gene expression of serotonin 1A (5HT_{1A}) receptors in human brain postmortem. *Brain Res.*, 658(1–2): 55–59.
- Martin-Ruiz, R., Puig, M.V., Celada, P., Shapiro, D.A., Roth, B.L., Mengod, G. and Artigas, F. (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J. Neurosci.*, 21(24): 9856–9866.
- Meador-Woodruff, J.H., Damask, S.P., Wang, J., Haroutunian, V., Davis, K.L. and Watson, S.J. (1996) Dopamine receptor mRNA expression in human striatum and neocortex. *Neuropsychopharmacology*, 15(1): 17–29.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21(Suppl. 2): 106S–115S.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27(7): 1159–1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251(1): 238–246.
- Mengod, G., Vilaró, M.T., Landwehrmeyer, G.B., Martínez-Mir, M.I., Niznik, H.B., Sunahara, R.K., Seeman, P., O'Dowd, B.F., Probst, A. and Palacios, J.M. (1992) Visualization of dopamine D₁, D₂ and D₃ receptor mRNAs in human and rat brain. *Neurochem. Int.*, 20(Suppl.): 33S–43S.
- Mengod, G., Vilaró, M.T., Raurich, A., López-Giménez, J.F., Cortés, R. and Palacios, J.M. (1996) 5-HT receptors in mammalian brain: receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. *Histochem. J.*, 28(11): 747–758.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. and Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, 78(1): 189–225.
- Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R. and Goldman-Rakic, P.S. (1996) Localization of dopamine D₄ receptors in GABAergic neurons of the primate brain. *Nature*, 381(6579): 245–248.
- Muly, E.C.I., Szigeti, K. and Goldman-Rakic, P.S. (1998) D₁ receptor in interneurons of macaque prefrontal cortex: distribution and subcellular localization. *J. Neurosci.*, 18(24): 10553–10565.
- Negyessy, L. and Goldman-Rakic, P.S. (2005) Subcellular localization of the dopamine D₂ receptor and coexistence with the calcium-binding protein neuronal calcium sensor-1 in the primate prefrontal cortex. *J. Comp. Neurol.*, 488(4): 464–475.
- Newman-Tancredi, A., Assie, M.B., Leduc, N., Ormiere, A.M., Danty, N. and Cosi, C. (2005) Novel antipsychotics activate recombinant human and native rat serotonin 5-HT_{1A} receptors: affinity, efficacy and potential implications for treatment of schizophrenia. *Int. J. Neuropsychopharmacol.*, 8(3): 341–356.
- Palacios, J.M., Camps, M., Cortés, R. and Probst, A. (1988) Mapping dopamine receptors in the human brain. *J. Neural Transm. Suppl.*, 27: 227–235.
- Paspalas, C.D. and Goldman-Rakic, P.S. (2005) Presynaptic D₁ dopamine receptors in primate prefrontal cortex: target-specific expression in the glutamatergic synapse. *J. Neurosci.*, 25(5): 1260–1267.
- Pasqualetti, M., Nardi, I., Ladinsky, H., Marazziti, D. and Cassano, G.B. (1996) Comparative anatomical distribution of serotonin 1A, 1D alpha and 2A receptor mRNAs in human brain postmortem. *Mol. Brain Res.*, 39(1–2): 223–233.
- Pazos, A., Cortés, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.*, 346(2): 231–249.
- Pazos, A. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, 346(2): 205–230.
- Pazos, A., Probst, A. and Palacios, J.M. (1987a) Serotonin receptors in the human brain — III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience*, 21(1): 97–122.
- Pazos, A., Probst, A. and Palacios, J.M. (1987b) Serotonin receptors in the human brain — IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*, 21(1): 123–139.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12(2): 440–453.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.*, 23(1–2): 163–178.
- Puig, M.V., Celada, P., Diaz-Mataix, L. and Artigas, F. (2003) In vivo modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT_{2A} receptors: relationship to thalamocortical afferents. *Cereb. Cortex*, 13(8): 870–882.

- Raghanti, M.A., Stimpson, C.D., Marcinkiewicz, J.L., Erwin, J.M., Hof, P.R. and Sherwood, C.C. (2008) Differences in cortical serotonergic innervation among humans, chimpanzees, and macaque monkeys: a comparative study. *Cereb. Cortex*, 18(3): 584–597.
- Riad, M., Garcia, S., Watkins, K.C., Jodoin, N., Doucet, E., Langlois, X., el Mestikawy, S., Hamon, M. and Descarries, L. (2000) Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J. Comp. Neurol.*, 417(2): 181–194.
- Rollema, H., Lu, Y., Schmidt, A.W. and Zorn, S.H. (1997) Clozapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. *Eur. J. Pharmacol.*, 338(2): R3–R5.
- Sakaue, M., Somboonthum, P., Nishihara, B., Koyama, Y., Hashimoto, H., Baba, A. and Matsuda, T. (2000) Post-synaptic 5-hydroxytryptamine(1A) receptor activation increases in vivo dopamine release in rat prefrontal cortex. *Br. J. Pharmacol.*, 129(5): 1028–1034.
- Santana, N., Bortolozzi, A., Serrats, J., Mengod, G. and Artigas, F. (2004) Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex*, 14(10): 1100–1109.
- Seamans, J.K. and Yang, C.R. (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog. Neurobiol.*, 74(1): 1–58.
- Sesack, S.R., Deutch, A.Y., Roth, R.H. and Bunney, B.S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. *J. Comp. Neurol.*, 290(2): 213–242.
- Sesack, S.R. and Pickel, V.M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.*, 320(2): 145–160.
- Sesack, S.R., Snyder, C.L. and Lewis, D.A. (1995) Axon terminals immunolabeled for dopamine or tyrosine hydroxylase synapse on GABA-immunoreactive dendrites in rat and monkey cortex. *J. Comp. Neurol.*, 363(2): 264–280.
- Sibley, D.R. (1999) New insights into dopaminergic receptor function using antisense and genetically altered animals. *Annu. Rev. Pharmacol. Toxicol.*, 39: 313–341.
- Smiley, J.F. and Goldman-Rakic, P.S. (1996b) Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J. Comp. Neurol.*, 367(3): 431–443.
- Smiley, J.F., Levey, A.I., Ciliax, B.J. and Goldman-Rakic, P.S. (1994) D1 dopamine receptor immunoreactivity in human and monkey cerebral cortex: predominant and extrasynaptic localization in dendritic spines. *Proc. Natl. Acad. Sci. U.S.A.*, 91(12): 5720–5724.
- Steinbusch, H.W. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4): 557–618.
- Sumiyoshi, T., Stockmeier, C.A., Overholser, J.C., Dilley, G.E. and Meltzer, H.Y. (1996) Serotonin1A receptors are increased in postmortem prefrontal cortex in schizophrenia. *Brain Res.*, 708(1–2): 209–214.
- Sunahara, R.K., Guan, H.C., O'Dowd, B.F., Seeman, P., Laurier, L.G., Ng, G., George, S.R., Torchia, J., Van Tol, H.H. and Niznik, H.B. (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature*, 350(6319): 614–619.
- Taber, M.T., Das, S. and Fibiger, H.C. (1995) Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. *J. Neurochem.*, 65(3): 1407–1410.
- Vallone, D., Picetti, R. and Borrelli, E. (2000) Structure and function of dopamine receptors. *Neurosci. Biobehav. Rev.*, 24(1): 125–132.
- Volk, D.W., Austin, M.C., Pierri, J.N., Sampson, A.R. and Lewis, D.A. (2000) Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Arch. Gen. Psychiatry*, 57(3): 237–245.
- Weinberger, D.R. (1988) Schizophrenia and the frontal lobe. *Trends Neurosci.*, 11(8): 367–370.
- Werkman, T.R., Glennon, J.C., Wadman, W.J. and McCreary, A.C. (2006) Dopamine receptor pharmacology: interactions with serotonin receptors and significance for the aetiology and treatment of schizophrenia. *CNS Neurol. Disord. Drug Targets*, 5(1): 3–23.
- Williams, G.V. and Goldman-Rakic, P.S. (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature*, 376(6541): 572–575.
- Williams, S.M. and Goldman-Rakic, P.S. (1993) Characterization of the dopaminergic innervation of the primate frontal cortex using a dopamine-specific antibody. *Cereb. Cortex*, 3(3): 199–222.
- Williams, S.M. and Goldman-Rakic, P.S. (1998) Widespread origin of the primate mesofrontal dopamine system. *Cereb. Cortex*, 8(4): 321–345.
- Willins, D.L., Deutch, A.Y. and Roth, B.L. (1997) Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse*, 27(1): 79–82.
- Wilson, M.A. and Molliver, M.E. (1991a) The organization of serotonergic projections to cerebral cortex in primates: regional distribution of axon terminals. *Neuroscience*, 44(3): 537–553.
- Wilson, M.A. and Molliver, M.E. (1991b) The organization of serotonergic projections to cerebral cortex in primates: retrograde transport studies. *Neuroscience*, 44(3): 555–570.
- Xu, T. and Pandey, S.C. (2000) Cellular localization of serotonin(2A) (5HT(2A)) receptors in the rat brain. *Brain Res. Bull.*, 51(6): 499–505.
- Zaitsev, A.V., Gonzalez-Burgos, G., Povysheva, N.V., Kroner, S., Lewis, D.A. and Krimer, L.S. (2005) Localization of calcium-binding proteins in physiologically and morphologically characterized interneurons of monkey dorsolateral prefrontal cortex. *Cereb. Cortex*, 15(8): 1178–1186.

CHAPTER 6

Alterations of dopamine and serotonin transmission in schizophrenia

Gary Remington^{1,2,*}

¹*Department of Psychiatry, Faculty of Medicine, University of Toronto, Ont., Canada*

²*Medication Assessment Program for Schizophrenia (MAPS) Clinic, Schizophrenia Program, Centre for Addiction and Mental Health, Toronto, Ont., Canada*

Abstract: The present chapter outlines current thinking regarding serotonin and dopamine in schizophrenia. Each has individually been linked to theories regarding the illness' pathophysiology although the focus here is on their interactive role, a model that has driven drug development in the field for the last 10–15 years. With clozapine as a prototype, a new class of 'atypical' antipsychotics entered the clinical market, hinged predominantly on the notion that these agents were superior to conventional antipsychotics through their ratio of serotonin 5-HT₂/dopamine D₂ binding. This model has since been challenged both clinically and theoretically, but interest in serotonin–dopamine interactions remains high in the face of a broader conceptualization of schizophrenia's symptom domains, in combination with a shift in the perceived role of dopamine vis-à-vis these different clinical features. At present, there is particular interest in the 5-HT_{1A}, 5-HT_{2C}, 5-HT₆ and 5-HT₇ receptors as the search for improved pharmacological treatments for schizophrenia continues.

Keywords: Serotonin (5-HT); dopamine (DA); schizophrenia; neurotransmitters; interactions.

Schizophrenia, dopamine and serotonin

Schizophrenia

Schizophrenia represents one of psychiatry's most debilitating and costly illnesses. Affecting approximately 1% of the population, it is routinely first detected during late adolescence and early adulthood, with a lifelong course often associated with persistent clinical symptoms and poor functional outcome (American Psychiatric Association, 1994; Robinson et al., 2004). Evidence suggests that within five years of the illness' onset, less than 15%

of individuals have returned to their pre-morbid level of functioning (Robinson et al., 2004). Generally thought to be neurodevelopmental in its origins (Lewis and Levitt, 2002; Walker, 2002; Pantelis et al., 2005; Rapoport et al., 2005), there is also evidence of a neuroprogressive component in a subgroup of individuals (Waddington et al., 1998; Lieberman, 1999). While there is clearly a genetic component (Sullivan, 2008), the pathophysiology of schizophrenia remains poorly understood and to date there are no biological markers that have proven useful in making a diagnosis or predicting those at risk of developing the illness (Verdoux and Cougnard, 2006; Bender et al., 2007).

The clinical essence of schizophrenia, at least historically, has been captured in the so-called positive symptoms, which include delusions and

*Corresponding author. Tel.: +1 416 535 8501 Ext. 4750;
Fax: +1 416 979 4292; E-mail: gary_remington@camh.net

hallucinations. Most individuals with this illness manifest some combination of such symptoms, and their unusual and sometimes bizarre nature often makes these features the most disconcerting. However, in recent years, greater attention has been given to other domains, in particular neurocognitive and negative symptoms (Harvey et al., 2006) (Table 1). Cognitive deficits are common and adversely affect a broad range of functions, e.g. attention, concentration and memory (Green and Nuechterlein, 2004). Similarly, negative or deficit symptoms are diverse, including avolition, alogia and anhedonia (Blanchard and Cohen, 2006; Kimhy et al., 2006; Kirkpatrick and Fischer, 2006). There is a growing recognition that it is these domains that are most influential in compromising functional recovery (Greenwood et al., 2005).

Schizophrenia and dopamine

The introduction of chlorpromazine (CPZ) in the early 1950s revolutionized our understanding and treatment of schizophrenia. It represented the first effective treatment for schizophrenia and provided compelling evidence that this illness is, at least in part, biologically mediated (Deniker, 1989, 1990).

As with other illnesses, much of our understanding regarding schizophrenia and its pathophysiology has occurred indirectly, through either drugs mimicking symptoms of the illness or identification of effective treatment strategies and subsequent deconstruction of their pharmacology.

The finding of CPZ’s antipsychotic activity was serendipitous, and it was only over the next several decades that blockade of dopamine, in particular the D₂ receptor, was identified as playing a critical role in this effect (Carlsson et al., 1958; Carlsson, 1963; Van Rossum, 1967; Seeman et al., 1976). Over time, other lines of investigation such as post-mortem, pharmacological challenge and central/peripheral marker studies offered further support regarding dopamine’s role (Goto and Grace, 2007; Guillin et al., 2007), and out of this collective work evolved a model identifying schizophrenia as a disorder of hyperdopaminergic activity, one that is still endorsed, albeit with modifications (see Dopamine).

Schizophrenia and serotonin

Noting a similarity between the effects of lysergic acid diethylamide (LSD) and symptoms of schizophrenia, it was proposed in the early 1950s that

Table 1. Schizophrenia, dopamine, symptoms and side effects

Dopamine pathway	Symptom domains/ side effects	Clinical features	Receptors	Dopamine
Mesolimbic	Positive	Thought disorder, e.g. disorganization Thought content, e.g. paranoia Perceptual disturbances, e.g. hallucinations	D ₂ , D ₃	↑
	Negative	Amotivation, apathy Anhedonia		↓
Mesocortical	Cognitive	Neuro, e.g. attention, concentration, memory Executive function Social, e.g. face/emotion recognition Decision making	D ₁	↓
	Negative	Amotivation, apathy Anhedonia		↓
Nigrostriatal	Motor/negative/ cognitive	Antipsychotic-related extrapyramidal symptoms (EPS) Secondary negative and cognitive symptoms	D ₂	Postsynaptic receptor blockade
Tuberoinfundibular	Endocrine	Antipsychotic-related hyperprolactinemia	D ₂	Postsynaptic receptor blockade

serotonergic activity may be decreased ('serotonin deficiency hypothesis') in schizophrenia (Gaddum, 1954; Woolley and Shaw, 1954). While clinical similarities existed, there were also notable differences that limited the applicability of this model (Breier, 1995).

However, other lines of investigation, employing strategies similar to those used to investigate dopamine, also suggested a role for serotonin (5-HT) in schizophrenia (Bleich et al., 1988; Breier, 1995; Roth and Meltzer, 1995; Abi-Dargham, 2007). In addition, 5-HT has been implicated in features of schizophrenia other than psychosis, e.g. anxiety, affect and cognition (Aghajanian and Sanders-Bush, 2002), offering yet further evidence that it is involved in at least some facets of the illness.

Summary

Since the 1960s, the role of dopamine has dominated thinking regarding schizophrenia and shaped three decades of antipsychotic development, culminating in the evolution of high-potency, selective D₂ antagonists as the mainstay in pharmacological treatment, e.g. haloperidol. This direction also reflected the field's focus on the positive symptoms of schizophrenia, one that only began to shift in the 1980s with a clearer distinction between positive and negative features, e.g. avolition and anhedonia.

Clozapine, serotonin–dopamine antagonism and the 'atypical' antipsychotics

Clozapine

Clozapine shifted thinking away from monolithic models of schizophrenia premised on either dopamine or 5-HT. Developed in the 1960s and introduced into clinical practice over the next decade, its widespread use was rapidly curtailed by a cluster of deaths in the early 1970s, later identified to be the result of the drug's risk of agranulocytosis. However, within this relatively short period, clozapine had positioned itself as unique clinically. It did not carry the risk of extrapyramidal symptoms (EPS) that had become

the hallmark of typical antipsychotic side effects, nor was it associated with hyperprolactinemia (Hippius, 1999). Evidence that it was superior in those who had not done well on typical antipsychotics, as well as beneficial in the treatment of negative symptoms, further underscored its uniqueness among available antipsychotics (Kane et al., 1988).

Clozapine and serotonin–dopamine antagonism

Pharmacologically, clozapine was at odds with the direction antipsychotic development had taken. Efforts had moved towards developing highly selective D₂ antagonists although it was soon apparent that these agents were not a clinical panacea, calling into question a purely dopaminergic model (Schmidt et al., 1995). In contrast, clozapine had a heterogeneous receptor binding profile, more in keeping with low-potency antipsychotics such as CPZ (Coward et al., 1989).

Notable in clozapine's pharmacological profile was its 5-HT₂ binding affinity, and it became the prototype of a new class of 'atypical', 'novel' or 'second-generation' antipsychotics (SGAs). The model was premised on the notion that it was this aspect of clozapine that accounted for its unique, atypical clinical properties (i.e. diminished EPS liability, greater efficacy in refractory schizophrenia and broader spectrum of clinical response). Meltzer et al. (1989), using pK_i values for striatal D₁, D₂ and frontal cortex 5-HT₂ receptors in rat brain, calculated ratios to predict the atypicality of a number of compounds based on pre-clinical and clinical data. A 5-HT₂/D₂ ratio of 1.19 (25-fold selectivity for 5-HT₂ vs. D₂) or higher correctly matched 92% (35/38) of the compounds examined.

Inherent to this notion is *greater* 5-HT₂ versus D₂ antagonism. Various typical antipsychotics exhibit 5-HT₂, in addition to D₂, binding, e.g. loxapine (Meltzer et al., 1989; Remington and Kapur, 1999). However, a ratio less than 1.19 does not translate to atypical clinical properties.

'Atypical' antipsychotics

Development of putative antipsychotics embraced this model, hoping to capture clozapine's clinical

benefits by incorporating this pharmacological feature while at the same time circumventing its troublesome side effects, in particular the risk of agranulocytosis. Marketed as 5-HT–dopamine antagonists (SDAs), the list included risperidone, olanzapine, quetiapine, sertindole, ziprasidone and zotepine (Fleischhacker and Hummer, 1997; Emsley and Oosthuizen, 2004). Over the next years, claims of their clinical superiority versus typical antipsychotics expanded to such areas as cognition, affect and functional outcome (Remington, 2003).

It is important to note that atypical antipsychotics are not confined to SDAs. Drugs like amisulpride (Leucht et al., 2002) demonstrated that atypicality could be achieved through other approaches, ‘limbic selectivity’ (Stephenson et al., 2000; Xiberas et al., 2001a, b; Nyberg et al., 2002; Bressan et al., 2003a, b) and ‘fast-off’ D₂ receptors (Seeman and Tallerico, 1998, 1999; Kapur and Seeman, 2001) representing two alternative models, while more recently aripiprazole claimed atypical status based on its profile of partial dopamine agonist activity (McGavin and Goa, 2002; Grady et al., 2003; Gupta and Masand, 2004; Kessler, 2007). However, through the 1990s, the field was dominated by the SDAs and the purported benefits of 5-HT₂/D₂ antagonism.

Summary

Clozapine fundamentally challenged the position that the ideal antipsychotic is characterized by highly selective D₂ antagonism. Its superior clinical profile, in the face of relatively low binding affinity for D₂ receptors, argued for an effect through different mechanisms, and it was posited that the clinical advantages related to its profile of greater 5-HT₂ versus D₂ antagonism.

By the 1990s, antipsychotic development was dominated by this model and enthusiasm for 5-HT’s role led to the notion that perhaps selective 5-HT₂ antagonists, e.g. MDL 100907 and ritan-serin, would prove to be effective antipsychotics and, in so doing, avoid the significant side effects inherent in compounds that also blocked dopamine. It turned out not to be the case, as alone they were not effective antipsychotics, but this did not

diminish the notion that combined 5-HT₂/D₂ activity is associated with distinct clinical advantages.

Dopamine and serotonin: neurobiology

Dopamine

Dopaminergic neurons arise from the mesencephalon or midbrain and project to the forebrain via three pathways (Goto and Grace, 2007; Guillin et al., 2007). The *nigrostriatal* path arises in the substantia nigra pars compacta and projects to the dorsal striatum (caudate–putamen). This system has been primarily implicated in the modulation of motor behaviour and its blockade by antipsychotics is tied to EPS and secondary negative symptoms that have been inextricably linked to these agents (Carpenter et al., 1988; Casey, 1991). The *mesolimbic* path consists of dopaminergic neurons arising in the ventral tegmental area (VTA) and projecting to the ventral striatum (nucleus accumbens). It is this system that is most closely associated with the positive symptoms of schizophrenia and implicated in ‘antipsychotic’ response per se (Crow et al., 1977). In addition, it has been linked to goal-directed behaviours through the modulation of motivation and reward (Mogenson et al., 1980). The *mesocortical* pathway represents dopaminergic neurons also arising in the VTA and projecting to cortical regions; these projections have garnered attention regarding their potential role in the cognitive, as well as negative, features of schizophrenia (Weinberger, 1987; Weinberger et al., 1988; Abi-Dargham, 2003). The *tuberoinfundibular* pathway warrants comment as antipsychotics’ influence here is linked to the hyperprolactinemia observed with all typical and at least some atypical antipsychotics (Moore, 1987; Haddad and Wieck, 2004). This path arises from dopaminergic cells situated in the arcuate nucleus and periventricular nuclei, with terminals in the medial eminence on the ventral surface of the hypothalamus, and it is responsible for the release of hypothalamic-releasing factors into the anterior pituitary.

There are five identified dopamine receptors (D_1 – D_5), and these have been categorized within two families (D_1 : D_1 and D_5 ; and D_2 : D_2 , D_3 and D_4) based on their genetic homology and common second messenger systems (Gingrich and Caron, 1993; Sokoloff et al., 1995; Guillin et al., 2007). D_1 and D_2 receptors are prominent in cortical regions and striatum, respectively, while D_3 and D_4 receptors have a higher distribution in the limbic system. D_5 receptors are concentrated in the hippocampal region. Historically, the greatest attention has been given to the D_2 receptor, for it appears that D_2 blockade is essential for antipsychotic activity (Seeman et al., 1976; Gingrich and Caron, 1993; Sokoloff et al., 1995; Kapur and Remington, 2001; Guillin et al., 2007). D_3 receptors have stimulated interest as a putative site of antipsychotic action given their concentration in the ventral striatum, but pursuit of this line of investigation has been hampered by the lack of selective ligands (Sokoloff et al., 1990; Micheli and Heidbreder, 2006). The localization of the D_1 receptor to cortical regions has led to speculation that it may be involved in the cognitive and negative symptoms of schizophrenia (Weinberger, 1987; Abi-Dargham, 2003; El-Ghundi et al., 2007). There has been speculation that clozapine's relatively high affinity for the D_4 receptor may account, at least in part, for its unique clinical attributes although evidence in support of this has not been forthcoming (Wong and Van Tol, 2003). At present, the role of the D_5 receptor in schizophrenia has not been well established.

Several other aspects of the dopaminergic system should be highlighted in the context of current thinking regarding schizophrenia. The most widely held model at present no longer conceptualizes schizophrenia as simply a disorder of hyperdopaminergic activity. Dopamine's role is now viewed in the context of more complex neurocircuitry, with disruption in feedback loops leading to an imbalance between cortical and subcortical dopamine systems (Weinberger, 1987; Weinberger et al., 1988; Abi-Dargham, 2002, 2004, 2007). Specifically, it has been postulated that prefrontal dopaminergic activity may play an inhibitory role in modulating dopamine at the level of subcortical structures. Further, a

hypodopaminergic state at the cortical level compromises this effect, resulting in hyperdopaminergic activity in subcortical regions that is manifested clinically as the positive symptoms. In contrast, decreased dopaminergic activity at the cortical level is implicated in the cognitive and negative symptom domains. Such a shift in thinking has important theoretical and treatment implications since it confers different roles for dopamine and its respective receptors in the broader clinical profile presently used to conceptualize schizophrenia.

This shift has been integrated with current thinking regarding dopamine's activity at a cellular level. Dopamine neurons exhibit two spike firing patterns (Grace and Bunney, 1984a, b; Goto and Grace, 2007). Tonic spike firing represents baseline spontaneous activity, while burst spike firing is sensitive to external stimuli, e.g. stress (Grace and Bunney, 1984a; Schultz et al., 1993). It has been postulated that schizophrenia may be characterized by decreased tonic activity, manifested clinically in the form of negative and cognitive symptoms (Guillin et al., 2007). This decrease in tonic activity, in turn, results in hypersensitivity of the dopamine system to phasic release, accounting for the positive symptoms. Although the two systems are thought to work in concert, they are mediated by different mechanisms. For example, from the standpoint of 5-HT receptor subtypes, it appears that the 5-HT_{2C} receptor is unique in its capacity to influence (i.e. inhibit) tonic dopamine release (Alex and Pehek, 2007).

Finally, recent advances have afforded the opportunity to better investigate the D_2 receptor in its low- and high-affinity states, the latter representing the functional physiological state (McDonald et al., 1984; George et al., 1985). Multiple drugs and non-pharmacological interventions have been found to increase the high-affinity states of D_2 receptors; to date, work involving selective serotonergic influences has been limited, with ketanserin not producing an increase (Seeman et al., 2006). This line of investigation will undoubtedly expand and has clear implications for an illness like schizophrenia where current evidence indicates that some degree of D_2 blockade is required for antipsychotic response (Seeman

et al., 1976; McDonald et al., 1984; George et al., 1985; Kapur and Remington, 2001).

Serotonin

Serotonergic neurons also arise from the midbrain, with the dorsal raphe nucleus projecting to cortex and striatal regions while the median raphe nucleus project to different limbic regions (Abi-Dargham, 2007; Alex and Pehek, 2007; Marek, 2007). Based on genetic sequences and second messenger systems, 7 distinct families of 5-HT receptors have been identified (5-HT₁–5-HT₇) with at least 15 subpopulations (Glennon et al., 1995; Aghajanian and Sanders-Bush, 2002). One of these subpopulations, 5-HT_{1P}, has not been identified in the central nervous system (CNS). The receptor families can be grouped as follows: ion-gated channel signal transduction (5-HT₃); G-protein-mediated signal transduction (5-HT_{1A/1B/1D/1E/1F}, 5-HT₄, 5-HT₆ and 5-HT₇); and phosphoinositol-mediated signal transduction (5-HT_{2A/2B/2C}).

The multiplicity of 5-HT receptors, widespread distribution through the CNS and links to a variety of behaviours make it difficult to clearly establish which of these receptors are most relevant to schizophrenia. Clozapine, with its unique clinical profile even among other atypical antipsychotics, has a high affinity for at least five 5-HT receptors: 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT₆ and 5-HT₇ (Coward et al., 1989; Abi-Dargham, 2007). In development of the SGAs, considerable attention was devoted to the role of the 5-HT_{2A} receptor. Autoradiographic evidence indicates that 5-HT_{2A/2C} receptors are located in 5-HT terminal regions and are particularly dense in the prefrontal and anterior cingulate cortices (Pazos et al., 1985; Fischette et al., 1987). The 5-HT_{2C} receptor garnered attention, in part, because of data suggesting that this aspect of atypical antipsychotics' pharmacology may account for the considerable weight gain liability observed in a number of these agents (Tecott et al., 1995; Ellingrod et al., 2005; Miller et al., 2005). The pharmacological profile of atypical agents such as clozapine, ziprasidone, aripiprazole and bifenox has raised interest in the putative role of the 5-HT_{1A} receptor (i.e. 5-HT_{1A} agonism) (Abi-Dargham,

2007; Newman-Tancredi et al., 2007), and while there appears to be a lack of 5-HT_{1A} receptors in either the substantia nigra or the dorsal striatum, these receptors are rich in the raphe where they serve as somatodendritic autoreceptors (Pazos and Palacios, 1985; Pompeiano et al., 1992). Clozapine's affinity for both the 5-HT₆ and the 5-HT₇ receptors has led to speculation that they may play a role in its clinical superiority, particularly cognition (Meltzer et al., 2003; Abi-Dargham, 2007). Evidence indicates that 5-HT₆ neurons are identifiable in dopamine terminal areas that include frontal cortex, hippocampus, nucleus accumbens and striatum (Gerard et al., 1996, 1997), while 5-HT₇ receptors can be found in cortex, hippocampus and amygdala (Neumaier et al., 2001).

Summary

Schizophrenia is now conceptualized as an illness with multiple symptom domains. The neurobiology of dopamine and 5-HT in the CNS positions each to play numerous roles in schizophrenia's pathophysiology, and the complexity of the two systems implicates a range of possible interactive mechanisms. From the standpoint of drug development, questions regarding the nature of their involvement extend beyond the action of antipsychotics to side effects as well for in clinical practice this takes on almost as much importance as therapeutic efficacy.

Serotonin–dopamine interactions

Serotonergic innervation of dopamine pathways

In general terms, raphe stimulation inhibits the activity of dopaminergic neurons (Abi-Dargham, 2007). On closer examination though, serotonergic receptors have different and apparently opposing effects on dopamine; moreover, their mechanism of action may differ as a function of multiple factors, including receptor subtype (Lucas and Spampinato, 2000), region (Arborelius et al., 1993), dose (in the case of pharmacological probes) (Goldstein et al., 1989), route/duration of

administration (Minabe et al., 2001) and concomitant receptor activity (Seeman et al., 1976; Goldstein et al., 1989; Andersson et al., 1995; Liegeois et al., 2002).

5-HT interactions will be discussed as they pertain to the three dopaminergic systems implicated in EPS (nigrostriatal), positive (mesolimbic), negative and cognitive (mesocortical) symptoms. Readers are referred to several excellent and recent reviews on this topic (Meltzer et al., 2003; Abi-Dargham, 2007; Alex and Pehek, 2007; Marek, 2007).

Nigrostriatal dopamine

The 5-HT_{1A} receptor has been implicated in dopamine cell activity, but not release (Alex and Pehek, 2007). The latter has been further confirmed in vivo in humans, using positron emission tomography (PET), with evidence that administration of a selective 5-HT_{1A} agonist, flesinoxan, does not alter striatal [¹¹C]raclopride binding (Bantick et al., 2005). A second PET study involving psilocybin reported decreased [¹¹C]raclopride binding in both the caudate and the putamen (19 and 20%, respectively), but this compound has mixed 5-HT_{1A} and 5-HT_{2A} agonist properties (Vollenweider et al., 1999). PET data with citalopram, a selective 5-HT uptake inhibitor, have also reported decreased [¹¹C]raclopride binding in both caudate and putamen although this does not shed light on the role of different receptors (Tiihonen et al., 1996). Evidence involving the 5-HT_{1B} receptor is conflicting (Ng et al., 1999; Sarhan et al., 1999).

The 5-HT₂ receptor appears involved in the tonic release of dopamine, thought to be reflected by extracellular dopamine (Grace, 1991); increases have been reported in the nucleus accumbens with ritanserin (Devaud and Hollingsworth, 1991). This has been demonstrated in vivo as well, using PET to examine baboons following administration of altanserin and SR 46349B (Dewey et al., 1995). Each of these agents shares in common 5-HT_{2A} antagonist properties, although effects on tonic dopamine release appear related to their 5-HT_{2C} antagonism (Lucas and Spampinato, 2000; Lucas et al., 2000a, b; Alex and Pehek, 2007).

Administration of 5-HT_{2C} agonists has been shown to decrease striatal dopamine, suggesting an inhibitory role for 5-HT_{2C} receptors in this regard (De Deurwaerdere et al., 2004), while administration of an antagonist or inverse agonist results in increases (De Deurwaerdere and Spampinato, 1999; De Deurwaerdere et al., 2004).

The 5-HT_{2A} receptor also appears involved in evoked dopamine release, for example, methylsergide, mianserin and cianserin antagonize haloperidol-induced striatal dopamine turnover (Waldmeier and Delini-Stula, 1979). Similarly, SR 46349B and the more selective 5-HT_{2A} antagonist MDL 100907 block increased dopamine in the striatum following administration of haloperidol and methamphetamine, respectively (Waldmeier and Delini-Stula, 1979; Schmidt et al., 1994; Lucas and Spampinato, 2000).

As well as their role in tonic inhibition, 5-HT_{2C} receptors have been reported to play a role in phasic release of dopamine in the nigrostriatum. Increased phasic dopamine release is observed with 5-HT_{2C} inverse agonists and attenuated by agonists (Porrás et al., 2002b; Navailles et al., 2004). This said, there is also evidence that has called into question the impact of 5-HT_{2C} receptors in the nigrostriatal system (Di Matteo et al., 2002; Di Giovanni et al., 2006).

5-HT₃ and 5-HT₄ receptors are not implicated in tonic dopamine activity, but appear to facilitate phasic dopamine release (Porrás et al., 2002a, 2003; Essock et al., 2006).

At present, any role for 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2B}, 5-HT₅, 5-HT₆ or 5-HT₇ receptors in dopamine activity has yet to be established.

Finally, 5-HT's modulation of dopamine in the nigrostriatal system may, in part, be mediated by both γ -aminobutyric acid (GABA) and acetylcholine (ACh) (Davies and Tongroach, 1978; Giambalvo and Snodgrass, 1978; James and Starr, 1980; Yamamoto et al., 1995; Boothman et al., 2006).

Mesolimbic dopamine

The 5-HT_{1A} receptor appears to have a stimulatory effect on mesolimbic dopamine. Activation of 5-HT_{1A} autoreceptors does not alter basal dopamine activity but decreases locomotion, in line

with attenuated dopamine release (Carey et al., 2004), while stimulation of the postsynaptic 5-HT_{1A} receptor increases cocaine-induced locomotion, thought to reflect stimulated dopamine release (De La Garza and Cunningham, 2000; Carey et al., 2004). 5-HT_{1B} receptors also facilitate dopamine release in this region through an interactive role with GABA (Parsons et al., 1999; O'Dell and Parsons, 2004).

The 5-HT₂ antagonists ritanserin and ICS 169369 have both been shown to have a greater effect on VTA versus substantia nigra dopamine release, although this difference is lost at higher doses (Goldstein et al., 1989). Both basal and burst firings are increased in a dose-dependent fashion with administration of the mixed 5-HT_{2A/2C} agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-amino-propane] (DOI), attenuated in each case by MDL 100907, a selective 5-HT_{2A} antagonist (Bortolozzi et al., 2005). Of note, local application of DOI in the medial prefrontal cortex (PFC) increases both basal and burst dopamine cell firings in the VTA, supporting the argument that VTA dopamine neurons are, at least in part, under the excitatory control of 5-HT_{2A} receptors in the medial PFC (Bortolozzi et al., 2005). As in the nigrostriatal system, 5-HT_{2C} receptors appear to be involved in the tonic regulation of dopamine (Dremencov et al., 2005) and, once more, this seems GABA-mediated though other mechanisms have been suggested (Di Giovanni et al., 2001; Ji et al., 2006). While the other 5-HT receptors have a stimulatory effect in this region, activation of 5-HT_{2C} receptors decreases firing rate of dopamine neurons, in contrast to inverse agonists and antagonists which demonstrate an opposite effect (Esposito, 2006). These same agents potentiate cocaine-induced dopamine increases, establishing the role of the 5-HT_{2C} receptors in the modulation of phasic dopamine release in the mesolimbic system (Navailles et al., 2004).

5-HT₃ receptors do not appear involved in basal dopamine release, but do seem to facilitate phasic dopamine release, e.g. with cocaine (McNeish et al., 1993; Kankaanpää et al., 2002). As of yet, the 5-HT₄ receptor has not been linked to tonic or phasic modulation of dopamine in this region.

Mesocortical dopamine

Evidence suggests that the 5-HT_{1A} receptor has a stimulatory effect on dopamine in the PFC, as 5-HT_{1A} agonists increase dopamine, which can be blocked by a 5-HT_{1A} antagonist (Rollema et al., 2000). Clozapine demonstrates 5-HT_{1A} agonist properties, and its stimulatory effect on mesocortical dopamine release (Morrow et al., 1999) is attenuated with WAY 100635 and *p*-MMPI, 5-HT_{1A} antagonists (Rollema et al., 1997; Hagino and Watanabe, 2002). This has led to the suggestion that clozapine's stimulation of 5-HT_{1A} somatodendritic autoreceptors in the dorsal raphe nucleus leads to increased dopamine in the PFC (Hagino and Watanabe, 2002). The 5-HT_{1B} receptor has also been implicated, as local application of 5-HT_{1B} agonists increases prefrontal dopamine, which is blocked by 5-HT_{1B} antagonists (Iyer and Bradberry, 1996).

Basal dopamine activity is not influenced by 5-HT_{2A} receptors, although they are involved in phasic dopamine release. For example, the selective 5-HT_{2A} antagonist MDL 100907 attenuates fluoxetine-induced increases in dopamine release (Zhang et al., 2000). Concomitant administration of 5-HT₂ antagonists, including MDL 100907, potentiates cortical dopamine release in the context of D₂ antagonism (Andersson et al., 1995; Liegeois et al., 2002), an important issue from the standpoint of antipsychotic drug development. It appears that 5-HT_{2C} receptors tonically inhibit cortical dopamine release, although this is mediated through their effect on VTA 5-HT_{2C} receptors (Di Matteo et al., 2002; Alex and Pehek, 2007). They also appear involved in phasic cortical dopamine release although, once again, this does not seem to involve cortical 5-HT_{2C} receptors (Pozzi et al., 2002; Alex et al., 2005; Pehek et al., 2006).

The 5-HT₃ receptor does appear to have an influence on PFC dopamine; for example, antidepressant-induced dopamine release in the PFC is decreased with local application of the 5-HT₃ antagonist ICS 205-930 (Tanda et al., 1995). However, the precise nature of this relationship requires clarification (Alex and Pehek, 2007). To date, evidence is lacking for a role

involving 5-HT₄, 5-HT₅, 5-HT₆ and 5-HT₇ receptors.

As with other dopamine pathways, there is evidence that additional neurotransmitters, including glutamate and GABA, may be involved in 5-HT's modulation of cortical dopamine (Pehek et al., 2006; Di Pietro and Seamans, 2007; Fink and Gothert, 2007).

Summary

There is clear evidence that 5-HT can modulate dopamine regionally in ways that might translate to clinical improvement in negative and cognitive symptoms (mesocortical) or EPS (nigrostriatal). From a theoretical perspective, these features share in common the potential benefit of enhanced dopamine activity. While this can be achieved through different serotonergic receptors, there is an added level of complexity related to regional differences in the distribution of these receptors and their actions. In addition, there are data to indicate that other neurotransmitters may be involved in 5-HT's modulation of dopamine.

Serotonin–dopamine: pre-clinical behavioural evidence

In drug development, there are a number of pre-clinical behavioural measures that have been used to evaluate putative antipsychotics (Geyer and Ellenbroek, 2003; Hagan and Jones, 2005; van den Buuse et al., 2005). The optimal model allows for an evaluation of potential therapeutic response versus side effects across a range of doses, with the goal of achieving the widest separation possible between antipsychotic potential and side effects. With the typical antipsychotics, the focus soon became one of antipsychotic potential versus risk of EPS, the latter inextricably linked to the first generation of antipsychotics. However, as the conceptualization of schizophrenia broadened to include other symptom domains, so too have efforts that would allow pre-clinical evaluation of these features (i.e. negative and cognitive symptoms). The following section, while not exhaustive, briefly reviews the different domains from the

standpoint of serotonergic/dopaminergic modulation.

Psychosis (positive symptoms)

At least four measures appear to represent viable models: conditioned avoidance response (CAR) (i.e. blockade of response in an aversive stimulus paradigm); apomorphine-induced climbing; dopamine agonist-induced locomotor activity; and the paw test [prolongation of forelimb and hindlimb reaction time (FRT, HRT)]. In the paw test, FRT is thought to reflect nucleus accumbens (EPS) and HRT neostriatal involvement (antipsychotic activity) (Ellenbroek et al., 1987; Ellenbroek and Cools, 1988).

5-HT's potential benefit has been demonstrated with each of these different measures. Typical and atypical antipsychotics prolong HRT equally, while atypicals have less impact on FRT (Ellenbroek et al., 1987, 1996; Cools et al., 1995). Along similar lines, haloperidol's effect is decreased by the 5-HT_{1A} agonist 8-OHDAPT and the 5-HT₂ antagonists ketanserin and ritanserin (Ellenbroek et al., 1994), as well as the 5-HT_{2A} inverse agonist ACP-103 (Gardell et al., 2007). Risperidone's attenuation of MK-801-induced hyperlocomotion [an *N*-methyl-D-aspartate (NMDA) antagonist] is blocked with ritanserin, a 5-HT_{2A/2C} antagonist (Su et al., 2007). By the same token, MDL 100907 and amperozide, also 5-HT_{2A} antagonists, decrease amphetamine-induced hyperactivity (Schmidt et al., 1995). When administered alone, CP-809-101 and WAY 163909, each selective 5-HT_{2C} agonists, mirror the profile of atypical antipsychotics in inhibiting CAR without notable catalepsy (Marquis et al., 2007; Siuciak et al., 2007).

In general though, these tests do not distinguish between typical and atypical antipsychotics, including the SDAs. This is not so surprising since antipsychotic activity is central to all putative antipsychotics, while benefits in other areas may or may not be evident. These findings are also in keeping with clinical evidence that fails to substantiate notable differences in antipsychotic activity between the older and the newer agents, excepting clozapine (see Positive symptoms).

EPS

Measures include catalepsy (rigidity/decreased pain) and paw test, both models applicable to rodents, as well as dystonia (monkeys). It has been pointed out that reduced liability of EPS across therapeutic doses represents the sine qua non of 'atypical' antipsychotics (Meltzer, 1995); thus, the expectation of superiority over typical antipsychotics on these measures represents a fundamental assumption.

Serotonergic agonists such as 5-hydroxytryptophan (a precursor of 5-HT) and quipazine (a direct acting agonist) worsen haloperidol-induced catalepsy (Balsara et al., 1979; Fuenmayor and Vogt, 1979). Conversely, 5-HT_{1A} agonists, through their action on autoreceptors, decrease cataleptogenic effects secondary to D₂ blockade (Kleven et al., 2005; Bardin et al., 2007), whereas blockade of 5-HT_{1A} receptors, e.g. with WAY 100635, can unmask or exacerbate pre-existing catalepsy (Bardin et al., 2006). Similarly, 5-HT_{1A} agonists such as 8-OH-DPAT and buspirone counteract tacrine-induced tremulous jaw movements in rats, a model for Parkinsonian tremor, an effect that again can be blocked by WAY 100635 (Zazpe et al., 2006). The combination of a selective serotonin reuptake inhibitor (SSRI), in combination with WAY 100635, produces antipsychotic-like activity in CAR and catalepsy, both of which can be suppressed with the administration of SB 242084, a selective 5-HT_{2C} antagonist (Eltayb et al., 2007). It has been reported that SSRIs alone can attenuate antipsychotic-induced catalepsy (Pires et al., 2005), although this is at odds with clinical data that acknowledge a risk for EPS with SSRIs (Leo, 1996).

The central role ascribed to 5-HT₂ receptors in the SDAs culminated in the development of selective 5-HT₂ antagonists, with the hope that these agents could act as 'stand-alone antipsychotics' devoid of D₂ antagonism and associated side effects. Pre-clinical evidence regarding the role of 5-HT₂ blockade is, however, not straightforward. Administration of 5-HT_{2A} antagonists, e.g. ritanserin, MDL 100907 and amperozide, has been shown to decrease catalepsy in some reports (Balsara et al., 1979; Fuenmayor and Vogt, 1979;

Hicks, 1990; Neal-Beliveau et al., 1993; Schmidt et al., 1995), but not others (Arnt and Bach-Lauritsen, 1986; Wadenberg, 1992). Evidence from primate models evaluating acute dystonia, a variant of EPS, has also proven conflicting. SDAs have been shown to differ in risk of dystonia in such models, with risperidone, olanzapine and ziprasidone sharing this risk with haloperidol in antipsychotic-primed animals, in contrast to clozapine and quetiapine where liability was minimal (Schmidt et al., 1995). These differences have raised the issue of a dose-dependent risk (Kapur and Remington, 1996), as well as speculation that other mechanisms of action may be involved in this effect (Kapur and Remington, 2001; Seeman, 2002; Remington, 2003).

Negative symptoms

Animal models such as CAR for antipsychotic potential and catalepsy for EPS risk are now well accepted as pre-clinical measures for putative antipsychotics. As of yet, the same cannot be said for models of negative symptoms and this may be related to several factors. It is only more recently that attention has turned to the importance of other symptom domains, and in terms of negative symptoms, it was initially postulated that these reflected morphological changes and, therefore, would not be amenable to pharmacotherapy (Crow, 1980). Clinically, distinguishing between primary (also called 'deficit') and secondary negative symptoms is difficult and those features that constitute true deficit symptoms still remain the subject of debate (Kirkpatrick et al., 2006).

Animal models to date have focused on two specific components, anhedonia and social withdrawal (Ellenbroek and Cools, 1990). While various measures remain the subject of investigation, the one test that has now been incorporated to some extent is that of social isolation in monkeys induced by amphetamine or phencyclidine (PCP) (Ellenbroek and Cools, 1990; Sams-Dodd, 1996; Geyer and Ellenbroek, 2003). In the case of amphetamine-induced isolation, it appears that typical antipsychotics fail to reverse this effect (Miczek and Yoshimura, 1982), although this is not the case for atypicals such as quetiapine and

clozapine (Ellenbroek et al., 1996). Results are less clear with PCP-induced social isolation, possibly reflecting the fact that these two drugs invoke a different clinical profile, implicating different mechanisms of action.

Cognition

As with negative symptoms, work in this area is comparatively recent. However, interest is intense because of evidence suggesting it is these other features that represent the rate-limiting step in functional recovery for those with schizophrenia (Green, 1996; Greenwood et al., 2005). Different facets to cognition exist and, in the context of pre-clinical measures, at least seven have been identified: working memory; attention/vigilance; verbal learning and memory; visual learning and memory; speed of processing; reasoning and problem solving; and social cognition (Hagan and Jones, 2005). Proposed pre-clinical tests range from maze tasks (working memory; reasoning and problem solving) to five-choice serial reaction time tasks (attention/vigilance; speed of processing).

Of the various measures, it is pre-pulse inhibition (PPI) that has garnered the most attention in evaluating the potential cognitive benefits of antipsychotics and dissecting mechanisms of action. The model is premised on the notion that deficits in sensory gating result in the cognitive abnormalities routinely observed in those with schizophrenia (Braff and Geyer, 1990). The measure involves the administration of a low-intensity, non-startling stimulus (usually acoustic), which attenuates the magnitude of subsequent startle response to a strong and startling stimulus. It is a model that has, as a distinct advantage, applicability at the human level since PPI deficits can be identified in schizophrenia (Geyer, 2006).

There are variants to the PPI model in animals based on induction of disruption and postulated underlying mechanisms. For example, disruption in PPI can be observed with the following: agonists, e.g. dopamine, 5-HT and noradrenaline; antagonists, e.g. NMDA; and rearing in isolation (Geyer and Ellenbroek, 2003; Barr et al., 2006). Any interpretation of results must also be viewed in the context of the model employed (Ojima et al.,

2004). PPI clearly taps into complex processes mediated by different mechanisms, and current evidence indicates that not all agents claiming atypical status on other measures such as CAR and catalepsy attenuate or reverse PPI deficits (Geyer and Ellenbroek, 2003; Auclair et al., 2006).

Dopamine agonist disruption of PPI fails to distinguish differences between typical and atypical antipsychotics, while disruption with serotonergic agonists such as DOI is more sensitive to antipsychotics with 5-HT_{2A} antagonist activity (Geyer and Ellenbroek, 2003). Further, it has been shown that DOI-induced PPI disruption is sensitive to MDL 100907 but not haloperidol (Schmidt et al., 1995). As an aside, MDL 100907 has also been shown to attenuate PPI deficits observed in dopamine transporter (DAT) knockout mice (Barr et al., 2004), in addition to deficits in attention and executive control, as measured using a five-choice serial reaction time task, produced by NMDA receptor antagonism (Mirjana et al., 2004).

The exact relationship between 5-HT and dopamine in mediating PPI is not clear. Pre-treatment with aripiprazole, but not clozapine, olanzapine or risperidone, can reduce the effect of 8-OH-DPAT-induced PPI disruption (van den Buuse and Gogos, 2007). Selective 5-HT_{2C} agonists, e.g. WAY 163909 and CP-809-101, have been shown to attenuate not only DOI-disrupted PPI, but also apomorphine and MK-801 disruptions, implicating interactions with both dopamine and glutamate (Marquis et al., 2007; Siuciak et al., 2007). Further work has implicated a role for the 5-HT_{1A} receptor, highlighting as well though that a balance between D₂ and 5-HT_{1A} activity may be critical to this effect (Park et al., 2005; Auclair et al., 2006). PPI disruptions with both apomorphine and LSD are not attenuated with either 5-HT₆ (Ro 04-6790) or 5-HT₇ antagonists (Ro 65-7199) (Leng et al., 2003).

Summary

Numerous lines of investigation, drawing upon a variety of pre-clinical behavioural measures, substantiate claims of 5-HT–dopamine interactions in the CNS that could impact on schizophrenia and its various symptoms. These findings offer further

theoretical support for the SDAs, which over the last decade have supplanted the typical antipsychotics in clinical practice. There are now considerable data that address the potential clinical benefits of these newer agents and the opportunity to evaluate such claims represents a necessary and critical final stage in establishing whether theory has effectively translated to clinical practice.

Serotonin–dopamine: clinical evidence

Since the 1990s, numerous SDAs have entered the market (see ‘Atypical’ Antipsychotics), and we now have both efficacy and effectiveness data that allow a comparative evaluation of this new class of antipsychotics with their typical counterparts.

In addition, there are more limited data arising from the investigation of selective 5-HT₂ antagonists and SSRIs that shed light on EPS and negative symptoms. These findings will also be reviewed here.

Positive symptoms

Clozapine’s superior efficacy in refractory schizophrenia led to speculation that these newer SDAs will demonstrate improved control of positive symptoms. Refractory schizophrenia and positive symptoms are not necessarily synonymous, although it is often assumed that positive symptoms represent the crux of treatment resistance (Remington et al., 2005).

Initial regulatory data involving efficacy trials suggested that other SDAs share clozapine’s benefits in this regard; however, evidence since has not substantiated this claim (Remington and Kapur, 2000; McEvoy et al., 2006; Lewis and Lieberman, 2008). What these results have demonstrated is that clozapine remains unique, even among the other atypical antipsychotics, in the treatment of refractory schizophrenia. This finding has important theoretical implications, for it indicates that mirroring clozapine’s profile of greater 5-HT₂ versus D₂ antagonism is not sufficient to mimic its clinical effects in this particular population.

EPS

Clozapine clearly demonstrated superiority in this regard (Peacock et al., 1996), and data from the efficacy trials for subsequent SDAs substantiated this same clinical benefit (Gao et al., 2008). However, it is also evident that while greater 5-HT₂ versus D₂ antagonism is shared in common by each of these drugs, they are not equal with respect to EPS liability. In contrast to clozapine, for example, risperidone demonstrates a dose-dependent increase in EPS that makes it comparable to conventional antipsychotics at higher doses (Chouinard et al., 1993; Marder and Meibach, 1994; Kapur et al., 1995). In vivo neuroimaging with PET has shed considerable light on this issue, establishing that risk of EPS increases markedly with D₂ occupancy levels above 80% (Farde et al., 1992; Kapur et al., 2000a). Even at higher doses, drugs like clozapine and quetiapine do not approximate this threshold, suggesting that their decreased risk of EPS across therapeutic doses might simply be explained by this feature rather than concomitant 5-HT₂ antagonism (Kapur et al., 1999, 2000b). That olanzapine has a D₂ profile more in keeping with risperidone, but a comparatively lower risk of EPS, can be explained by its inherent anticholinergic activity, a feature not observed in risperidone (Raedler et al., 2000). Finally, the degree of difference between typical antipsychotics and SDAs in terms of EPS has been challenged based on several methodological issues, including frequent use of haloperidol as the comparator, a high-potency typical antipsychotic with a marked liability for EPS (Leucht et al., 2003). In addition, it has often been used at high doses, further increasing its EPS risk (Geddes et al., 2000).

The work with selective 5-HT₂ antagonists favours the positive results arising from shorter term, controlled efficacy trials with the SDAs. Setoperone has been shown to diminish antipsychotic-related EPS (Ceulemans et al., 1985), as has ritanserin (Reyntjens et al., 1986; Gelders, 1989; Bersani et al., 1990), which has also been linked to improvement in akathisia (Miller et al., 1990, 1992) in addition to tremor and akinesia linked to Parkinson’s disease (Hildebrand and Delecluse,

1987; Henderson et al., 1992). In contrast, SSRIs have been shown to induce EPS (Leo, 1996), although the risk is notably less than what is observed with conventional antipsychotics.

Negative symptoms

In the seminal work that led to clozapine's reintroduction in many countries following haematologic concerns, it proved superior to conventional antipsychotics, in this case CPZ, for control of negative symptoms (Kane et al., 1988). It had been argued previously that the negative symptoms, e.g. amotivation and anhedonia, reflect morphological changes that do not make them amenable to pharmacological treatment (Crow, 1980), so these favourable findings with clozapine fundamentally shifted expectations regarding the new generation of antipsychotics.

Results suggested benefits for the SDAs in this regard, although such findings have been modest and inconsistent (Buckley and Stahl, 2007). Further, significant methodological concerns related to trial design have tempered these results. The major focus of this criticism has rested on the dosing of the typical antipsychotics used for comparison, identified as excessively high based on both clinical and neuroimaging data (Baldessarini et al., 1988; Kapur et al., 1997, 2000a; Geddes et al., 2000; Carpenter and Conley, 2007). It has been argued that the adverse side effects associated with D₂ antagonism, which can mirror the negative symptoms (Voruganti et al., 2001; de Haan et al., 2003, 2004, 2005; Verhoeff et al., 2003; Saeedi et al., 2006; Voruganti and Awad, 2006), could instead account for the benefits being ascribed to the SDAs.

Paralleling these concerns was the proposal that purported benefits could also be accounted for by the altered dopaminergic profiles of these newer antipsychotics versus typical agents. Specifically, it was postulated that rapid dissociation from the D₂ receptor offered a viable alternative explanation, challenging the notion that concomitant 5-HT₂ antagonism played a role in these benefits (Seeman and Talerico, 1998; Kapur and Seeman, 2001; Seeman, 2002, 2006). Moreover, it was noted that other drugs, e.g. aripiprazole and amisulpride,

without the pharmacological profile of SDAs, shared the same clinical benefits (Leucht et al., 2002; Gupta and Masand, 2004; El-Sayeh et al., 2006).

The addition of setoperone or ritanserin, 5-HT₂ antagonists, has been associated with improvement in negative and affective symptoms in those with schizophrenia (Ceulemans et al., 1985; Reyntjens et al., 1986; Reyntjens, 1986; Duinkerke et al., 1993). In contrast, a recent meta-analysis failed to support claims that SSRIs are useful as an augmentation strategy in the management of negative symptoms (Sepehry et al., 2007).

Cognitive symptoms

In many ways, the cognitive story has mirrored that observed for negative symptoms. Favourable results, albeit modest, were reported for clozapine and other SDAs, but these findings were challenged on a number of the same methodological issues (Marder, 2006a, b; Carpenter and Conley, 2007; Goldberg et al., 2007). Subsequent trials employing lower doses of the comparative typical antipsychotics, in keeping with more recent guidelines, have failed to corroborate these differences (Green et al., 2002).

As this field has advanced, attention has shifted from neurocognition to incorporate social cognition as well (i.e. how individuals integrate, process and respond to surrounding social cues). Those with schizophrenia have identified deficits in this realm (Bertrand et al., 2007; Sergi et al., 2007b), but to date the benefits of antipsychotics, including the SDAs, have been inconsistent (Savina and Beninger, 2007; Sergi et al., 2007a).

Summary

Initial enthusiasm that accompanied the introduction of the SDAs has been tempered. First, the greater 5-HT₂ versus D₂ antagonism characterized by clozapine's pharmacology does not appear to account for its unique clinical profile, as evidenced by the fact that other SDAs do not match its therapeutic efficacy in refractory schizophrenia. Second, in the 'real world' of effectiveness versus efficacy, the SDAs have fallen short with respect to

early claims of clinical superiority across various symptom domains. Any such superiority, if present, is modest. For health care systems strained by fiscal demands, such clinical data must be balanced against the substantially higher costs of these newer drugs, as well as the recognition that they too have their own troublesome side effects. In the case of the SDAs, purported benefits in terms of EPS have been traded for an increased risk of weight gain and metabolic disturbances in this class of antipsychotics, raising the challenge as to whether we have simply exchanged one serious side effect for another.

Future directions

Antipsychotic development has clearly been influenced by the aforementioned findings. Already there has been a shift in focus away from such SDA-like agents, reflected in the development of partial dopamine agonists (Tamminga, 2002; Gupta and Masand, 2004; Kessler, 2007) and dopamine stabilizers (Tamminga, 2002; Gupta and Masand, 2004; Carlsson and Carlsson, 2006; Kessler, 2007). At the same time, the search for other pharmacological strategies continues (Miyamoto et al., 2005).

The role of 5-HT and its interaction with dopamine continues to hold promise for a number of reasons. Benefits of the SDAs, modest as they may be, cannot be ignored in an illness as debilitating as schizophrenia. Moreover, preliminary evidence suggesting a diminished risk of tardive dyskinesia (Correll et al., 2004; Kane, 2006; Remington, 2007), if substantiated with longer term data, represents a major advance over typical antipsychotics. To date, attention at the clinical level has largely been confined to the potential for concomitant 5-HT₂ antagonism; however, there is ample evidence to support the pursuit of other 5-HT receptors and this is already underway (Meltzer et al., 2003; Miyamoto et al., 2005; Abi-Dargham, 2007; Gray and Roth, 2007). The complexity of the serotonergic system is well recognized, but significant advances are continuously being made and the development of more site-selective agonists and antagonists will offer

greater opportunity to tease apart the nuances of 5-HT–dopamine interactions. Similarly, ongoing efforts to better delineate the features of negative and cognitive symptoms clinically will permit more specific measures to evaluate putative treatments.

Evidence that the SDAs do not mirror clozapine's clinical benefits has provided important information and does not diminish an approach that tries to deconstruct clozapine's mechanisms of action. Placing this strategy in the context of what we know regarding distribution and neurobiology of the different receptors, in addition to current theories regarding the pathophysiology of schizophrenia's symptom domains, can accelerate this process. For example, clozapine demonstrates partial 5-HT_{1A} agonist properties and other atypical antipsychotics share this feature, e.g. olanzapine, quetiapine, ziprasidone and bifenox (Abi-Dargham, 2007; Newman-Tancredi et al., 2007). Both basic and pre-clinical evidence suggest that such activity could, through modulation of dopamine, decrease EPS liability while improving negative and cognitive symptoms. Clozapine and other atypicals demonstrate 5-HT_{2C} blocking properties, and pursuit of its potential benefits is warranted based on evidence that 5-HT_{2C} antagonists can increase dopamine in both the nucleus accumbens and the PFC (Di Matteo et al., 1998, 2002). Clozapine's high affinity for the 5-HT₆ and 5-HT₇ receptors, in combination with existing knowledge regarding their regional distribution, encourages further work along these lines as well. Indeed, a selective 5-HT₆ antagonist, GW742457, is already under investigation in psychosis and Alzheimer's disease (Phase I, unpublished data). Table 2 represents a summary of the pre-clinical evidence for these selected receptors, as well as their current clinical status.

Finally, while the focus here has been on 5-HT–dopamine interactions, there is considerable evidence implicating the role of other neurotransmitters in this process, providing for other lines of approach. Various reports have been noted, for example, where 5-HT's modulation of dopamine appears GABA-mediated (Giambalvo and Snodgrass, 1978; Yamamoto et al., 1995; Di Giovanni et al., 2001).

Table 2. Summary of current evidence for selected serotonergic receptors

Mechanism	Clozapine	Current evidence	Selective clinical agent(s)	Current status
5-HT _{1A} agonism	++	Enhanced mesocorticolimbic DA function	Tandospirone	Various lines of investigation, e.g. anxiety, cognition
5-HT _{2A} antagonism	+++	Enhanced mesocorticolimbic DA function Nigrostriatal – mixed results	Ritanserin (2A/2B/2C) MDL 100907	Ritanserin-failed trials as ‘antipsychotic’ MDL 100907-failed trials as ‘antipsychotic’
5-HT _{2C} antagonism	+++	Enhanced mesocorticolimbic DA function Role in tonic/phasic DA activity	SR 46349B	Unpublished data
5-HT ₆ antagonism	+++	Evidence lacking	SB-271046	No data
5-HT ₇ antagonism	+++	Evidence lacking	No	N/A

The climate in which this work will continue is notably different from that a decade ago when SDAs entered the clinical market. Functional, versus clinical, outcome has taken on increased importance and it now appears that the cognitive and negative symptoms represent the rate-limiting step in functional recovery (Green, 1996; Greenwood et al., 2005). With evidence that these features are identifiable in the illness’ prodrome and well in advance of psychotic symptoms (Kraepelin, 1971; Keefe et al., 2006; Pukrop et al., 2007), a fundamental question facing this research relates to whether pharmacotherapy can substantively alter these core symptoms.

The limited gains of atypical antipsychotics, including the SDAs, has resulted in less emphasis on a ‘magic bullet’ compound that addresses all symptoms and greater acceptance of the position that multiple pharmacological strategies may be required, shaped to meet the individual’s clinical profile. This has shifted attention away from the development of single compounds with heterogeneous receptor binding profiles to more selective agents, targeting specific symptoms, which can be used as add-on therapies. The impact of this shift is already evident — numerous targets for cognitive enhancement have been identified, including various selective serotonergic compounds (Gray and Roth, 2007). Regulatory bodies appear more prepared to embrace such a strategy.

For the first time, the United States Food and Drug Administration (FDA) has indicated that it would consider approving such add-on therapies (Laughren and Levin, 2006). At the same time, various putative antipsychotics under investigation maintain a multi-receptor approach that includes serotonergic activity beyond 5-HT₂ antagonism, e.g. bifeprunox (Newman-Tancredi et al., 2007).

It is very likely that future gains in our understanding of schizophrenia and its treatment, at least for the foreseeable future, will reflect small but incremental advances and the identified potential benefits arising from 5-HT’s interaction with dopamine ensure continued investigation along these lines. At the very least, this work will advance our understanding of the illness’ pathophysiology, as each has been implicated. Ideally, gains will also be made in treatment although it remains as to whether modulation of dopamine can effect the magnitude of change that will translate to clinically relevant benefits.

Abbreviations

5-HT	serotonin
CAR	conditioned avoidance response
CNS	central nervous system
CPZ	chlorpromazine
D or DA	dopamine

DOI	1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]
EPS	extrapyramidal symptoms
FRT	forelimb reaction time
GABA	γ -aminobutyric acid
HRT	hindlimb reaction time
LSD	lysergic acid diethylamide
NMDA	<i>N</i> -methyl-D-aspartate
pK_i	negative log of K_i value, where $K_i = IC_{50}/(1 + L/K_D)$ with L the concentration and K_D the apparent dissociation constant of the 3H -ligand
PCP	phencyclidine
PET	positron emission tomography
PFC	prefrontal cortex
PPI	pre-pulse inhibition
SDA	serotonin–dopamine antagonist
SSRI	selective serotonin reuptake inhibitor
VTA	ventral tegmental area

References

- Abi-Dargham, A. (2002) Recent evidence for dopamine abnormalities in schizophrenia. *Eur. Psychiatry*, 17: 341–347.
- Abi-Dargham, A. (2003) Probing cortical dopamine function in schizophrenia: what can D_1 receptors tell us? *World Psychiatry*, 2: 166–171.
- Abi-Dargham, A. (2004) Do we still believe in the dopamine hypothesis? New data bring new evidence. *Int. J. Neuropsychopharmacol.*, 7(Suppl. 1): S1–S5.
- Abi-Dargham, A. (2007) Alterations of serotonin transmission in schizophrenia. *Int. Rev. Neurobiol.*, 78: 133–164.
- Aghajanian, G.K. and Sanders-Bush, E. (2002) Serotonin. In: Cheney D., Davis K.L., Coyle J.T. and Nemeroff C. (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress*. Williams & Wilkins, New York, NY, pp. 15–34.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113: 296–320.
- Alex, K.D., Yavarian, G.J., McFarlane, H.G., Pluto, C.P. and Pehek, E.A. (2005) Modulation of dopamine release by striatal 5-HT_{2C} receptors. *Synapse*, 55: 242–251.
- American Psychiatric Association. (1994) *Diagnostic and Statistical Manual of Mental Disorders* (4th edn.). American Psychiatric Association, Washington, DC.
- Andersson, J.L., Nomikos, G.G., Marcus, M., Hertel, P., Mathe, J.M. and Svensson, T.H. (1995) Ritanerlin potentiates the stimulatory effects of raclopride on neuronal activity and dopamine release selectivity in the mesolimbic dopaminergic system. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 352: 374–385.
- Arborelius, L., Nomikos, G.G., Hacksell, U. and Svensson, T.H. (1993) (*R*)-8-OH-DPAT preferentially increases dopamine release in rat medial prefrontal cortex. *Acta Physiol. Scand.*, 148: 465–466.
- Arnt, J.H.J. and Bach-Lauritsen, T. (1986) Further studies of the mechanisms behind scopolamine-induced reversal of antistereotypic and cataleptogenic effects of neuroleptics in rats. *Acta Pharmacol. Toxicol. (Copenh)*, 59: 319–324.
- Auclair, A.L., Kleven, M.S., Besnard, J., Depoortere, R. and Newman-Tancredi, A. (2006) Actions of novel antipsychotic agents on apomorphine-induced PPI disruption: influence of combined serotonin 5-HT_{1A} receptor activation and dopamine D₂ receptor blockade. *Neuropsychopharmacology*, 31: 1900–1909.
- Baldessarini, R.J., Cohen, B.M. and Teicher, M.H. (1988) Significance of neuroleptic dose and plasma level in the pharmacological treatment of psychoses. *Arch. Gen. Psychiatry*, 45: 79–91.
- Balsara, J.J., Jadhav, J.H. and Chandorkar, A.G. (1979) Effect of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy. *Psychopharmacology (Berl.)*, 62: 67–69.
- Bantick, R.A., De Vries, M.H. and Grasby, P.M. (2005) The effect of a 5-HT_{1A} receptor agonist on striatal dopamine release. *Synapse*, 57: 67–75.
- Bardin, L., Auclair, A., Kleven, M.S., Prinssen, E.P., Koek, W., Newman-Tancredi, A. and Depoortere, R. (2007) Pharmacological profiles in rats of novel antipsychotics with combined dopamine D₂/serotonin 5-HT_{1A} activity: comparison with typical and atypical conventional antipsychotics. *Behav. Pharmacol.*, 18: 103–118.
- Bardin, L., Kleven, M.S., Barret-Grevoz, C., Depoortere, R. and Newman-Tancredi, A. (2006) Antipsychotic-like vs cataleptogenic actions in mice of novel antipsychotics having D₂ antagonist and 5-HT_{1A} agonist properties. *Neuropsychopharmacology*, 31: 1869–1879.
- Barr, A.M., Lehmann-Masten, V., Paulus, M., Gainetdinov, R.R., Caron, M.G. and Geyer, M.A. (2004) The selective serotonin-2A receptor antagonist M100907 reverses behavioral deficits in dopamine transporter knockout mice. *Neuropsychopharmacology*, 29: 221–228.
- Barr, A.M., Powell, S.B., Markou, A. and Geyer, M.A. (2006) Iloperidone reduces sensorimotor gating deficits in pharmacological models, but not a developmental model, of disrupted prepulse inhibition in rats. *Neuropharmacology*, 51: 457–465.
- Bender, S., Weisbrod, M. and Resch, F. (2007) Which perspectives can endophenotypes and biological markers offer in the early recognition of schizophrenia? *J. Neural Transm.*, 114: 1199–1215.
- Bersani, G., Grisipini, A., Marini, S., Pasini, A., Valducci, M. and Ciani, N. (1990) 5-HT₂ antagonist ritanerlin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. *Clin. Neuropharmacol.*, 13: 500–506.

- Bertrand, M.C., Sutton, H., Achim, A.M., Malla, A.K. and Lepage, M. (2007) Social cognitive impairments in first episode psychosis. *Schizophr. Res.*, 95: 124–133.
- Blanchard, J.J. and Cohen, A.S. (2006) The structure of negative symptoms within schizophrenia: implications for assessment. *Schizophr. Bull.*, 32: 238–245.
- Bleich, A., Brown, S.L., Kahn, R. and van Praag, H.M. (1988) The role of serotonin in schizophrenia. *Schizophr. Bull.*, 14: 297–315.
- Boothman, L., Raley, J., Denk, F., Hirani, E. and Sharp, T. (2006) In vivo evidence that 5-HT_{2C} receptors inhibit 5-HT neuronal activity via a GABAergic mechanism. *Br. J. Pharmacol.*, 149: 861–869.
- Bortolozzi, A., Diaz-Mataix, L., Scorza, M.C., Celada, P. and Artigas, F. (2005) The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. *J. Neurochem.*, 95: 1597–1607.
- Braff, D.L. and Geyer, M.A. (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch. Gen. Psychiatry*, 47: 181–188.
- Breier, A. (1995) Serotonin, schizophrenia and antipsychotic drug action. *Schizophr. Res.*, 14: 187–202.
- Bressan, R.A., Erlandsson, K., Jones, H.M., Mulligan, R., Flanagan, R.J., Ell, P.J. and Pilowsky, L.S. (2003a) Is regionally selective D₂/D₃ dopamine occupancy sufficient for atypical antipsychotic effect? An in vivo quantitative [¹²³I]epidepride SPET study of amisulpride-treated patients. *Am. J. Psychiatry*, 160: 1413–1420.
- Bressan, R.A., Erlandsson, K., Jones, H.M., Mulligan, R.S., Ell, P.J. and Pilowsky, L.S. (2003b) Optimizing limbic selective D₂/D₃ receptor occupancy by risperidone: a [¹²³I]epidepride SPET study. *J. Clin. Psychopharmacol.*, 23: 5–14.
- Buckley, P.F. and Stahl, S.M. (2007) Pharmacological treatment of negative symptoms of schizophrenia: therapeutic opportunity or Cul-de-sac? *Acta Psychiatr. Scand.*, 115: 93–100.
- Carey, R.J., Depalma, G., Damianopoulos, E., Muller, C.P. and Huston, J.P. (2004) The 5-HT_{1A} receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. *Psychopharmacology (Berl.)*, 177: 46–54.
- Carlsson, A. and Carlsson, M.L. (2006) A dopaminergic deficit hypothesis of schizophrenia: the path to discovery. *Dialogues Clin. Neurosci.*, 8: 137–142.
- Carlsson, A., Lindqvist, M., Magnusson, T. and Waldeck, B. (1958) On the presence of 3-hydroxytyramine in brain. *Science*, 127: p. 471.
- Carlsson, A.L.M. (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxy-tyramine and normetanephrine in mouse brain. *Acta Pharmacol. Toxicol.*, 20: 140–144.
- Carpenter, W.T., Jr. and Conley, R.R. (2007) Challenge to atypical antipsychotic drug effect on cognition. *Am. J. Psychiatry*, 164: 1910–1911.
- Carpenter, W.T., Jr., Heinrichs, D.W. and Wagman, A.M. (1988) Deficit and nondeficit forms of schizophrenia: the concept. *Am. J. Psychiatry*, 145: 578–583.
- Casey, D.E. (1991) Extrapyramidal syndromes in nonhuman primates: typical and atypical neuroleptics. *Psychopharmacol. Bull.*, 27: 47–50.
- Ceulemans, D.L., Gelders, Y.G., Hoppenbrouwers, M.L., Reyntjens, A.J. and Janssen, P.A. (1985) Effect of serotonin antagonism in schizophrenia: a pilot study with setoperone. *Psychopharmacology (Berl.)*, 85: 329–332.
- Chouinard, G., Jones, B., Remington, G., Bloom, D., Addington, D., MacEwan, G.W., Labelle, A., Beauclair, L. and Arnott, W. (1993) A Canadian multicenter placebo-controlled study of fixed doses of risperidone and haloperidol in the treatment of chronic schizophrenic patients. *J. Clin. Psychopharmacol.*, 13: 25–40.
- Cools, A.R., Prinssen, E.P. and Ellenbroek, B.A. (1995) The olfactory tubercle as a site of action of neuroleptics with an atypical profile in the paw test: effect of risperidone, prothipendyl, ORG 5222, sertindole and olanzapine. *Psychopharmacology (Berl.)*, 119: 428–439.
- Correll, C.U., Leucht, S. and Kane, J.M. (2004) Lower risk for tardive dyskinesia associated with second-generation antipsychotics: a systematic review of 1-year studies. *Am. J. Psychiatry*, 161: 414–425.
- Coward, D.M., Imperato, A., Urwyler, S. and White, T.G. (1989) Biochemical and behavioural properties of clozapine. *Psychopharmacology (Berl.)*, 99(Suppl.): S6–S12.
- Crow, T.J. (1980) Positive and negative schizophrenic symptoms and the role of dopamine. *Br. J. Psychiatry*, 137: 383–386.
- Crow, T.J., Deakin, J.F. and Longden, A. (1977) The nucleus accumbens — possible site of antipsychotic action of neuroleptic drugs? *Psychol. Med.*, 7: 213–221.
- Davies, J. and Tongroach, P. (1978) Neuropharmacological studies on the nigro-striatal and raphe-striatal system in the rat. *Eur. J. Pharmacol.*, 51: 91–100.
- De Deurwaerdere, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24: 3235–3241.
- De Deurwaerdere, P. and Spampinato, U. (1999) Role of serotonin_{2A} and serotonin_{2B/2C} receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73: 1033–1042.
- de Haan, L., Booij, J., Lavalaye, J., van Amelsvoort, T. and Linszen, D. (2005) Subjective experiences during dopamine depletion. *Am. J. Psychiatry*, 162: p. 1755.
- de Haan, L., Lavalaye, J., van Bruggen, M., van Nimwegen, L., Booij, J., van Amelsvoort, T. and Linszen, D. (2004) Subjective experience and dopamine D₂ receptor occupancy in patients treated with antipsychotics: clinical implications. *Can. J. Psychiatry*, 49: 290–296.
- de Haan, L., van Bruggen, M., Lavalaye, J., Booij, J., Dingemans, P.M. and Linszen, D. (2003) Subjective experience and D₂ receptor occupancy in patients with recent-onset schizophrenia treated with low-dose olanzapine or haloperidol: a randomized, double-blind study. *Am. J. Psychiatry*, 160: 303–309.

- De La Garza, R., 2nd and Cunningham, K.A. (2000) The effects of the 5-hydroxytryptamine_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin on spontaneous activity, cocaine-induced hyperactivity and behavioral sensitization: a micro-analysis of locomotor activity. *J. Pharmacol. Exp. Ther.*, 292: 610–617.
- Deniker, P. (1989) From chlorpromazine to tardive dyskinesia (brief history of neuroleptics). *Psychiatr. J. Univ. Ottawa*, 14: 253–259.
- Deniker, P. (1990) The neuroleptics: a historical survey. *Acta Physiol. Scand.*, 82: 83–87.
- Devaud, L.L. and Hollingsworth, E.B. (1991) Effects of the 5-HT₂ receptor antagonist, ritanserin, on biogenic amines in the rat nucleus accumbens. *Eur. J. Pharmacol.*, 192: 427–429.
- Dewey, S.L., Smith, G.S., Logan, J., Alexoff, D., Ding, Y.S., King, P., Pappas, N., Brodie, J.D. and Ashby, C.R., Jr. (1995) Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis. *J. Neurosci.*, 15: 821–829.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) *m*-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin_{2C} receptors. *Neuroscience*, 103: 111–116.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006) Central serotonin_{2C} receptor: from physiology to pathology. *Curr. Top. Med. Chem.*, 6: 1909–1925.
- Di Matteo, V., Cacchio, M., Di Giulio, C. and Esposito, E. (2002) Role of serotonin_{2C} receptors in the control of brain dopaminergic function. *Pharmacol. Biochem. Behav.*, 71: 727–734.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1998) Selective blockade of serotonin_{2C/2B} receptors enhances dopamine release in the rat nucleus accumbens. *Neuropharmacology*, 37: 265–272.
- Di Pietro, N.C. and Seamans, J.K. (2007) Dopamine and serotonin interactions in the prefrontal cortex: insights on antipsychotic drugs and their mechanism of action. *Pharmacopsychiatry*, 40(Suppl. 1): S27–S33.
- Dremencov, E., Newman, M.E., Kinor, N., Blatman-Jan, G., Schindler, C.J., Overstreet, D.H. and Yadid, G. (2005) Hyperfunctionality of serotonin_{2C} receptor-mediated inhibition of accumbal dopamine release in an animal model of depression is reversed by antidepressant treatment. *Neuropharmacology*, 48: 34–42.
- Duinkerke, S.J., Botter, P.A., Jansen, A.A., van Dongen, P.A., van Haften, A.J., Boom, A.J., van Laarhoven, J.H. and Busard, H.L. (1993) Ritanserin, a selective 5-HT_{2/1C} antagonist, and negative symptoms in schizophrenia. A placebo-controlled double-blind trial. *Br. J. Psychiatry*, 163: 451–455.
- El-Ghundi, M., O'Dowd, B.F. and George, S.R. (2007) Insights into the role of dopamine receptor systems in learning and memory. *Rev. Neurosci.*, 18: 37–66.
- El-Sayeh, H.G., Morganti, C. and Adams, C.E. (2006) Aripiprazole for schizophrenia. Systematic review. *Br. J. Psychiatry*, 189: 102–108.
- Ellenbroek, B. and Cools, A.R. (1988) The paw test: an animal model for neuroleptic drugs which fulfils the criteria for pharmacological isomorphism. *Life Sci.*, 42: 1205–1213.
- Ellenbroek, B.A. and Cools, A.R. (1990) Animal models with construct validity for schizophrenia. *Behav. Pharmacol.*, 1: 469–490.
- Ellenbroek, B.A., Lubbers, L.J. and Cools, A.R. (1996) Activity of “seroquel” (ICI 204,636) in animal models for atypical properties of antipsychotics: a comparison with clozapine. *Neuropsychopharmacology*, 15: 406–416.
- Ellenbroek, B.A., Peeters, B.W., Honig, W.M. and Cools, A.R. (1987) The paw test: a behavioural paradigm for differentiating between classical and atypical neuroleptic drugs. *Psychopharmacology (Berl.)*, 93: 343–348.
- Ellenbroek, B.A., Prinssen, E.P. and Cools, A.R. (1994) The role of serotonin receptor subtypes in the behavioural effects of neuroleptic drugs. A paw test study in rats. *Eur. J. Neurosci.*, 6: 1–8.
- Ellingrod, V.L., Perry, P.J., Ringold, J.C., Lund, B.C., Bever-Stille, K., Fleming, F., Holman, T.L. and Miller, D. (2005) Weight gain associated with the -759C/T polymorphism of the 5HT_{2C} receptor and olanzapine. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 134: 76–78.
- Eltayb, A., Wadenberg, M.L., Schilström, B. and Svensson, T.H. (2007) Antipsychotic-like effect by combined treatment with citalopram and WAY 100635: involvement of the 5-HT_{2C} receptor. *Int. J. Neuropsychopharmacol.*, 10: 405–410.
- Emsley, R. and Oosthuizen, P. (2004) Evidence-based pharmacotherapy of schizophrenia. *Int. J. Neuropsychopharmacol.*, 7: 219–238.
- Esposito, E. (2006) Serotonin–dopamine interaction as a focus of novel antidepressant drugs. *Curr. Drug Targets*, 7: 177–185.
- Essock, S.M., Covell, N.H., Davis, S.M., Stroup, T.S., Rosenheck, R.A. and Lieberman, J.A. (2006) Effectiveness of switching antipsychotic medications. *Am. J. Psychiatry*, 163: 2090–2095.
- Farde, L., Nordström, A.L., Wiesel, F.A., Pauli, S., Halldin, C. and Sedvall, G. (1992) Positron emission tomographic analysis of central D₁ and D₂ dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch. Gen. Psychiatry*, 49: 538–544.
- Fink, K.B. and Gothert, M. (2007) 5-HT receptor regulation of neurotransmitter release. *Pharmacol. Rev.*, 59: 360–417.
- Fischette, C.T., Nock, B. and Renner, K. (1987) Effects of 5,7-dihydroxytryptamine on serotonin₁ and serotonin₂ receptors throughout the rat central nervous system using quantitative autoradiography. *Brain Res.*, 421: 263–279.
- Fleischhacker, W.W. and Hummer, M. (1997) Drug treatment of schizophrenia in the 1990s. Achievements and future possibilities in optimising outcomes. *Drugs*, 53: 915–929.
- Fuenmayor, L.D. and Vogt, M. (1979) The influence of cerebral 5-hydroxytryptamine on catalepsy induced by brain-amine depleting neuroleptics or by cholinomimetics. *Br. J. Pharmacol.*, 67: 309–318.

- Gaddum, J. (1954) Drugs antagonistic to 5-hydroxytryptamine. In: Wolstenholme G. (Ed.), Ciba Foundation Symposium on Hypertension. Little Brown and Co., Boston, MA, pp. 75–77.
- Gao, K., Kemp, D.E., Ganocy, S.J., Gajwani, P., Xia, G. and Calabrese, J.R. (2008) Antipsychotic-induced extrapyramidal side effects in bipolar disorder and schizophrenia: a systematic review. *J. Clin. Psychopharmacol.*, 28: 203–209.
- Gardell, L.R., Vanover, K.E., Pounds, L., Johnson, R.W., Barido, R., Anderson, G.T., Veinbergs, I., Dyssegaard, A., Brunmark, P., Tabatabaei, A., Davis, R.E., Brann, M.R., Hacksell, U. and Bonhaus, D.W. (2007) ACP-103, a 5-hydroxytryptamine_{2A} receptor inverse agonist, improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. *J. Pharmacol. Exp. Ther.*, 322: 862–870.
- Geddes, J., Freemantle, N., Harrison, P. and Bebbington, P. (2000) Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *BMJ*, 321: 1371–1376.
- Gelders, Y.G. (1989) Thymosthenic agents, a novel approach in the treatment of schizophrenia. *Br. J. Psychiatry*, 155(Suppl.): 33–36.
- George, S.R., Watanabe, M., Di Paolo, T., Falardeau, P., Labrie, F. and Seeman, P. (1985) The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. *Endocrinology*, 117: 690–697.
- Gerard, C., el Mestikawy, S., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23: 164–173.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 746: 207–219.
- Geyer, M.A. (2006) The family of sensorimotor gating disorders: comorbidities or diagnostic overlaps? *Neurotox. Res.*, 10: 211–220.
- Geyer, M.A. and Ellenbroek, B. (2003) Animal behavior models of the mechanisms underlying antipsychotic atypicality. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1071–1079.
- Giambalvo, C.T. and Snodgrass, S.R. (1978) Biochemical and behavioral effects of serotonin neurotoxins on the nigrostriatal dopamine system: comparison of injection sites. *Brain Res.*, 152: 555–566.
- Gingrich, J.A. and Caron, M.G. (1993) Recent advances in the molecular biology of dopamine receptors. *Annu. Rev. Neurosci.*, 16: 299–321.
- Glennon, R.A., Dukat, M. and Westkaemper, R.B. (1995) Serotonin receptor subtypes and ligands. In: Bloom F.E. and Kupfer D. (Eds.), *Neuropsychopharmacology: The Fourth Generation of Progress*. Williams & Wilkins, New York, NY, pp. 415–429.
- Goldberg, T.E., Goldman, R.S., Burdick, K.E., Malhotra, A.K., Lencz, T., Patel, R.C., Woerner, M.G., Schooler, N.R., Kane, D.G. and Robinson, D.G. (2007) Cognitive improvement after treatment with second-generation antipsychotic medications in first-episode schizophrenia: is it a practice effect? *Arch. Gen. Psychiatry*, 64: 1115–1122.
- Goldstein, J.M., Litwin, L.C., Sutton, E.B. and Malick, J.B. (1989) Effects of ICI 169,369, a selective serotonin₂ antagonist, in electrophysiological tests predictive of antipsychotic activity. *J. Pharmacol. Exp. Ther.*, 249: 673–680.
- Goto, Y. and Grace, A.A. (2007) The dopamine system and the pathophysiology of schizophrenia: a basic science perspective. *Int. Rev. Neurobiol.*, 78: 41–68.
- Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, 41: 1–24.
- Grace, A.A. and Bunney, B.S. (1984a) The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.*, 4: 2877–2890.
- Grace, A.A. and Bunney, B.S. (1984b) The control of firing pattern in nigral dopamine neurons: single spike firing. *J. Neurosci.*, 4: 2866–2876.
- Grady, M.A., Gasperoni, T.L. and Kirkpatrick, P. (2003) Aripiprazole. *Nat. Rev. Drug Discov.*, 2: 427–428.
- Gray, J.A. and Roth, B.L. (2007) Molecular targets for treating cognitive dysfunction in schizophrenia. *Schizophr. Bull.*, 33: 1100–1119.
- Green, M.F. (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? *Am. J. Psychiatry*, 153: 321–330.
- Green, M.F., Marder, S.R., Glynn, S.M., McGurk, S.R., Wirshing, W.C., Wirshing, D.A., Liberman, R.P. and Mintz, J. (2002) The neurocognitive effects of low-dose haloperidol: a two-year comparison with risperidone. *Biol. Psychiatry*, 51: 972–978.
- Green, M.F. and Nuechterlein, K.H. (2004) The MATRICS initiative: developing a consensus cognitive battery for clinical trials. *Schizophr. Res.*, 72: 1–3.
- Greenwood, K.E., Landau, S. and Wykes, T. (2005) Negative symptoms and specific cognitive impairments as combined targets for improved functional outcome within cognitive remediation therapy. *Schizophr. Bull.*, 31: 910–921.
- Guillin, O., Abi-Dargham, A. and Laruelle, M. (2007) Neurobiology of dopamine in schizophrenia. *Int. Rev. Neurobiol.*, 78: 1–39.
- Gupta, S. and Masand, P. (2004) Aripiprazole: review of its pharmacology and therapeutic use in psychiatric disorders. *Ann. Clin. Psychiatry*, 16: 155–166.
- Haddad, P.M. and Wieck, A. (2004) Antipsychotic-induced hyperprolactinaemia: mechanisms, clinical features and management. *Drugs*, 64: 2291–2314.
- Hagan, J.J. and Jones, D.N. (2005) Predicting drug efficacy for cognitive deficits in schizophrenia. *Schizophr. Bull.*, 31: 830–853.
- Hagino, Y. and Watanabe, M. (2002) Effects of clozapine on the efflux of serotonin and dopamine in the rat brain: the role of 5-HT_{1A} receptors. *Can. J. Physiol. Pharmacol.*, 80: 1158–1166.

- Harvey, P.D., Koren, D., Reichenberg, A. and Bowie, C.R. (2006) Negative symptoms and cognitive deficits: what is the nature of their relationship? *Schizophr. Bull.*, 32: 250–258.
- Henderson, J., Yiannikas, C. and Graham, J.S. (1992) Effect of ritanserin, a highly selective 5-HT₂ receptor antagonist, on Parkinson's disease. *Clin. Exp. Neurol.*, 29: 277–282.
- Hicks, P.B. (1990) The effect of serotonergic agents on haloperidol-induced catalepsy. *Life Sci.*, 47: 1609–1615.
- Hildebrand, J. and Delecluse, F. (1987) Effect of ritanserin, a selective serotonin-S₂ antagonist, on Parkinsonian rest tremor. *Curr. Ther. Res.*, 41: 298–300.
- Hippius, H. (1999) A historical perspective of clozapine. *J. Clin. Psychiatry*, 60(Suppl. 12): 22–23.
- Iyer, R.N. and Bradberry, C.W. (1996) Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 277: 40–47.
- James, T.A. and Starr, M.S. (1980) Rotational behaviour elicited by 5-HT in the rat: evidence for an inhibitory role of 5-HT in the substantia nigra and corpus striatum. *J. Pharm. Pharmacol.*, 32: 196–200.
- Ji, S.P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R. and Zhang, X. (2006) Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat. Med.*, 12: 324–329.
- Kane, J., Honigfeld, G., Singer, J. and Meltzer, H. (1988) Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch. Gen. Psychiatry*, 45: 789–796.
- Kane, J.M. (2006) Tardive dyskinesia circa 2006. *Am. J. Psychiatry*, 163: 1316–1318.
- Kankaanpää, A., Meririnne, E. and Seppälä, T. (2002) 5-HT₃ receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. *Psychopharmacology (Berl.)*, 159: 341–350.
- Kapur, S. and Remington, G. (1996) Serotonin–dopamine interaction and its relevance to schizophrenia. *Am. J. Psychiatry*, 153: 466–476.
- Kapur, S. and Remington, G. (2001) Dopamine D₂ receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. *Biol. Psychiatry*, 50: 873–883.
- Kapur, S., Remington, G., Zipursky, R.B., Wilson, A.A. and Houle, S. (1995) The D₂ dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. *Life Sci.*, 57: 103–107.
- Kapur, S. and Seeman, P. (2001) Does fast dissociation from the dopamine D₂ receptor explain the action of atypical antipsychotics? A new hypothesis. *Am. J. Psychiatry*, 158: 360–369.
- Kapur, S., Zipursky, R., Jones, C., Remington, G. and Houle, S. (2000a) Relationship between dopamine D₂ occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am. J. Psychiatry*, 157: 514–520.
- Kapur, S., Zipursky, R., Jones, C., Shammí, C.S., Remington, G. and Seeman, P. (2000b) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D₂ receptor occupancy. *Arch. Gen. Psychiatry*, 57: 553–559.
- Kapur, S., Zipursky, R., Roy, P., Jones, C., Remington, G., Reed, K. and Houle, S. (1997) The relationship between D₂ receptor occupancy and plasma levels on low dose oral haloperidol: a PET study. *Psychopharmacology (Berl.)*, 131: 148–152.
- Kapur, S., Zipursky, R.B. and Remington, G. (1999) Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am. J. Psychiatry*, 156: 286–293.
- Keefe, R.S., Perkins, D.O., Gu, H., Zipursky, R.B., Christensen, B.K. and Lieberman, J.A. (2006) A longitudinal study of neurocognitive function in individuals at-risk for psychosis. *Schizophr. Res.*, 88: 26–35.
- Kessler, R.M. (2007) Aripiprazole: what is the role of dopamine D₂ receptor partial agonism? *Am. J. Psychiatry*, 164: 1310–1312.
- Kimhy, D., Yale, S., Goetz, R.R., McFarr, L.M. and Malaspina, D. (2006) The factorial structure of the schedule for the deficit syndrome in schizophrenia. *Schizophr. Bull.*, 32: 274–278.
- Kirkpatrick, B., Fenton, W.S., Carpenter, W.T., Jr. and Marder, S.R. (2006) The NIMH-MATRICES consensus statement on negative symptoms. *Schizophr. Bull.*, 32: 214–219.
- Kirkpatrick, B. and Fischer, B. (2006) Subdomains within the negative symptoms of schizophrenia: commentary. *Schizophr. Bull.*, 32: 246–249.
- Kleven, M.S., Barret-Grevoz, C., Bruins Slot, L. and Newman-Tancredi, A. (2005) Novel antipsychotic agents with 5-HT_{1A} agonist properties: role of 5-HT_{1A} receptor activation in attenuation of catalepsy induction in rats. *Neuropharmacology*, 49: 135–143.
- Kraepelin, E. (1971) *Dementia Praecox and Paraphrenia*. Krieger Publishing Co., Melbourne, FL.
- Laughren, T. and Levin, R. (2006) Food and Drug Administration perspective on negative symptoms in schizophrenia as a target for a drug treatment claim. *Schizophr. Bull.*, 32: 220–222.
- Leng, A., Ouagazzal, A., Feldon, J. and Higgins, G.A. (2003) Effect of the 5-HT₆ receptor antagonists Ro04-6790 and Ro65-7199 on latent inhibition and prepulse inhibition in the rat: comparison to clozapine. *Pharmacol. Biochem. Behav.*, 75: 281–288.
- Leo, R.J. (1996) Movement disorders associated with the serotonin selective reuptake inhibitors. *J. Clin. Psychiatry*, 57: 449–454.
- Leucht, S., Pitschel-Walz, G., Engel, R.R. and Kissling, W. (2002) Amisulpride, an unusual “atypical” antipsychotic: a meta-analysis of randomized controlled trials. *Am. J. Psychiatry*, 159: 180–190.
- Leucht, S., Wahlbeck, K., Hamann, J. and Kissling, W. (2003) New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. *Lancet*, 361: 1581–1589.

- Lewis, D.A. and Levitt, P. (2002) Schizophrenia as a disorder of neurodevelopment. *Annu. Rev. Neurosci.*, 25: 409–432.
- Lewis, S. and Lieberman, J. (2008) CATIE and CUtLASS: can we handle the truth? *Br. J. Psychiatry*, 192: 161–163.
- Lieberman, J.A. (1999) Is schizophrenia a neurodegenerative disorder? A clinical and neurobiological perspective. *Biol. Psychiatry*, 46: 729–739.
- Liegeois, J.F., Ichikawa, J. and Meltzer, H.Y. (2002) 5-HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res.*, 947: 157–165.
- Lucas, G., De Deurwaerdere, P., Caccia, S. and Umberto, S. (2000a) The effect of serotonergic agents on haloperidol-induced striatal dopamine release in vivo: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology*, 39: 1053–1063.
- Lucas, G., De Deurwaerdere, P., Porras, G. and Spampinato, U. (2000b) Endogenous serotonin enhances the release of dopamine in the striatum only when nigro-striatal dopaminergic transmission is activated. *Neuropharmacology*, 39: 1984–1995.
- Lucas, G. and Spampinato, U. (2000) Role of striatal serotonin_{2A} and serotonin_{2C} receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. *J. Neurochem.*, 74: 693–701.
- Marder, S.R. (2006a) Drug initiatives to improve cognitive function. *J. Clin. Psychiatry*, 67(Suppl. 9): 31–35.
- Marder, S.R. (2006b) Initiatives to promote the discovery of drugs to improve cognitive function in severe mental illness. *J. Clin. Psychiatry*, 67(Suppl. 9): p. e03.
- Marder, S.R. and Meibach, R.C. (1994) Risperidone in the treatment of schizophrenia. *Am. J. Psychiatry*, 151: 825–835.
- Marek, G.J. (2007) Serotonin and dopamine interactions in rodents and primates: implications for psychosis and antipsychotic drug development. *Int. Rev. Neurobiol.*, 78: 165–192.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Jr., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]: a novel 5-hydroxytryptamine_{2C} receptor-selective agonist with preclinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.
- McDonald, W.M., Sibley, D.R., Kilpatrick, B.F. and Caron, M.G. (1984) Dopaminergic inhibition of adenylate cyclase correlates with high affinity agonist binding to anterior pituitary D₂ dopamine receptors. *Mol. Cell. Endocrinol.*, 36: 201–209.
- McEvoy, J.P., Lieberman, J.A., Stroup, T.S., Davis, S.M., Meltzer, H.Y., Rosenheck, R.A., Swartz, M.S., Perkins, D.O., Keefe, R.S., Davis, C.E., Severe, J. and Hsiao, J.K. (2006) Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am. J. Psychiatry*, 163: 600–610.
- McGavin, J.K. and Goa, K.L. (2002) Aripiprazole. *CNS Drugs*, 16: 779–786.
- McNeish, C.S., Svingos, A.L., Hitzemann, R. and Strecker, R.E. (1993) The 5-HT₃ antagonist zacopride attenuates cocaine-induced increases in extracellular dopamine in rat nucleus accumbens. *Pharmacol. Biochem. Behav.*, 45: 759–763.
- Meltzer, H. (1995) Atypical antipsychotic drugs. In: Kupfer D. and Bloom F.E. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, NY, pp. 1277–1286.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159–1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) The ratios of serotonin₂ and dopamine₂ affinities differentiate atypical and typical antipsychotic drugs. *Psychopharmacol. Bull.*, 25: 390–392.
- Micheli, F. and Heidbreder, C. (2006) Selective dopamine D₃ receptor antagonists: a review 2001–2005. *Recent Patents CNS Drug Discov.*, 1: 271–288.
- Miczek, K.A. and Yoshimura, H. (1982) Disruption of primate social behavior by D-amphetamine and cocaine: differential antagonism by antipsychotics. *Psychopharmacology (Berl.)*, 76: 163–171.
- Miller, C.H., Fleischhacker, W.W., Ehrmann, H. and Kane, J.M. (1990) Treatment of neuroleptic induced akathisia with the 5-HT₂ antagonist ritanserin. *Psychopharmacol. Bull.*, 26: 373–376.
- Miller, C.H., Hummer, M., Pycha, R. and Fleischhacker, W.W. (1992) The effect of ritanserin on treatment-resistant neuroleptic induced akathisia: case reports. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 16: 247–251.
- Miller, D.D., Ellingrod, V.L., Holman, T.L., Buckley, P.F. and Arndt, S. (2005) Clozapine-induced weight gain associated with the 5HT_{2C} receptor -759C/T polymorphism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 133: 97–100.
- Minabe, Y., Hashimoto, K., Watanabe, K.I. and Ashby, C.R. Jr. (2001) Acute and repeated administration of the selective 5-HT_{2A} receptor antagonist M100907 significantly alters the activity of midbrain dopamine neurons: an in vivo electrophysiological study. *Synapse*, 40: 102–112.
- Mirjana, C., Baviera, M., Invernizzi, R.W. and Baldacci, C. (2004) The serotonin 5-HT_{2A} receptors antagonist M100907 prevents impairment in attentional performance by NMDA receptor blockade in the rat prefrontal cortex. *Neuropsychopharmacology*, 29: 1637–1647.
- Miyamoto, S., Duncan, G.E., Marx, C.E. and Lieberman, J.A. (2005) Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol. Psychiatry*, 10: 79–104.
- Mogenson, G.J., Jones, D.L. and Yim, C.Y. (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol.*, 14: 69–97.

- Moore, K.E. (1987) Interactions between prolactin and dopaminergic neurons. *Biol. Reprod.*, 36: 47–58.
- Morrow, B.A., Rosenberg, S.J. and Roth, R.H. (1999) Chronic clozapine, but not haloperidol, alters the response of mesoprefrontal dopamine neurons to stress and clozapine challenges in rats. *Synapse*, 34: 28–35.
- Navailles, S., De Deurwaerdere, P., Porras, G. and Spampinato, U. (2004) In vivo evidence that 5-HT_{2C} receptor antagonist but not agonist modulates cocaine-induced dopamine outflow in the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 29: 319–326.
- Neal-Beliveau, B.S., Joyce, J.N. and Lucki, I. (1993) Serotonergic involvement in haloperidol-induced catalepsy. *J. Pharmacol. Exp. Ther.*, 265: 207–217.
- Neumaier, J.F., Sexton, T.J., Yracheta, J., Diaz, A.M. and Brownfield, M. (2001) Localization of 5-HT₇ receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. *J. Chem. Neuroanat.*, 21: 63–73.
- Newman-Tancredi, A., Cussac, D. and Depoortere, R. (2007) Neuropharmacological profile of bifeprunox: merits and limitations in comparison with other third-generation antipsychotics. *Curr. Opin. Investig. Drugs*, 8: 539–554.
- Ng, N.K., Lee, H.S. and Wong, P.T. (1999) Regulation of striatal dopamine release through 5-HT₁ and 5-HT₂ receptors. *J. Neurosci. Res.*, 55: 600–607.
- Nyberg, S., Chou, Y.H. and Halldin, C. (2002) Saturation of striatal D₂ dopamine receptors by clozapine. *Int. J. Neuropsychopharmacol.*, 5: 11–16.
- O'Dell, L.E. and Parsons, L.H. (2004) Serotonin_{1B} receptors in the ventral tegmental area modulate cocaine-induced increases in nucleus accumbens dopamine levels. *J. Pharmacol. Exp. Ther.*, 311: 711–719.
- Ojima, T., Ito, C., Sakurai, E., Sakurai, E., Watanabe, T. and Yanai, K. (2004) Effects of serotonin–dopamine antagonists on prepulse inhibition and neurotransmitter contents in the rat cortex. *Neurosci. Lett.*, 366: 130–134.
- Pantelis, C., Yucel, M., Wood, S.J., Velakoulis, D., Sun, D., Berger, G., Stuart, G.W., Yung, A., Phillips, L. and McGorry, P.D. (2005) Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. *Schizophr. Bull.*, 31: 672–696.
- Park, W.K., Jeong, D., Cho, H., Lee, S.J., Cha, M.Y., Pae, A.N., Choi, K.I., Koh, H.Y. and Kong, J.Y. (2005) KHA-761, a potent D₃ receptor antagonist with high 5-HT_{1A} receptor affinity, exhibits antipsychotic properties in animal models of schizophrenia. *Pharmacol. Biochem. Behav.*, 82: 361–372.
- Parsons, L.H., Koob, G.F. and Weiss, F. (1999) RU 24969, a 5-HT_{1B/1A} receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. *Synapse*, 32: 132–135.
- Pazos, A., Cortes, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin₂ receptors. *Brain Res.*, 346: 231–249.
- Pazos, A. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin₁ receptors. *Brain Res.*, 346: 205–230.
- Peacock, L., Solgaard, T., Lublin, H. and Gerlach, J. (1996) Clozapine versus typical antipsychotics. A retro- and prospective study of extrapyramidal side effects. *Psychopharmacology (Berl.)*, 124: 188–196.
- Pehek, E.A., Nocjar, C., Roth, B.L., Byrd, T.A. and Mabrouk, O.S. (2006) Evidence for the preferential involvement of 5-HT_{2A} serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. *Neuropsychopharmacology*, 31: 265–277.
- Pires, J.G., Bonikowski, V. and Futuro-Neto, H.A. (2005) Acute effects of selective serotonin reuptake inhibitors on neuroleptic-induced catalepsy in mice. *Braz. J. Med. Biol. Res.*, 38: 1867–1872.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12: 440–453.
- Porras, G., De Deurwaerdere, P., Moison, D. and Spampinato, U. (2003) Conditional involvement of striatal serotonin₃ receptors in the control of in vivo dopamine outflow in the rat striatum. *Eur. J. Neurosci.*, 17: 771–781.
- Porras, G., Di Matteo, V., De Deurwaerdere, P., Esposito, E. and Spampinato, U. (2002a) Central serotonin₄ receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: in vivo studies with morphine, amphetamine and cocaine. *Neuropharmacology*, 43: 1099–1109.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E. and Spampinato, U. (2002b) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26: 311–324.
- Pozzi, L., Acconcia, S., Ceglia, I., Invernizzi, R.W. and Samanin, R. (2002) Stimulation of 5-hydroxytryptamine 5-HT_{2C} receptors in the ventro tegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J. Neurochem.*, 82: 93–100.
- Pukrop, R., Ruhrmann, S., Schultze-Lutter, F., Bechdolf, A., Brockhaus-Dumke, A. and Klosterkotter, J. (2007) Neurocognitive indicators for a conversion to psychosis: comparison of patients in a potentially initial prodromal state who did or did not convert to a psychosis. *Schizophr. Res.*, 92: 116–125.
- Raedler, T.J., Knable, M.B., Jones, D.W., Lafargue, T., Urbina, R.A., Egan, M.F., Pickar, D. and Weinberger, D.R. (2000) In vivo olanzapine occupancy of muscarinic acetylcholine receptors in patients with schizophrenia. *Neuropsychopharmacology*, 23: 56–68.
- Rapoport, J.L., Addington, A.M., Frangou, S. and Psych, M.R. (2005) The neurodevelopmental model of schizophrenia: update 2005. *Mol. Psychiatry*, 10: 434–449.
- Remington, G. (2003) Understanding antipsychotic “atypicality”: a clinical and pharmacological moving target. *J. Psychiatry Neurosci.*, 28: 275–284.

- Remington, G. (2007) Tardive dyskinesia: eliminated, forgotten, or overshadowed? *Curr. Opin. Psychiatry*, 20: 131–137.
- Remington, G. and Kapur, S. (1999) D₂ and 5-HT₂ receptor effects of antipsychotics: bridging basic and clinical findings using PET. *J. Clin. Psychiatry*, 60(Suppl. 10): 15–19.
- Remington, G. and Kapur, S. (2000) Atypical antipsychotics: are some more atypical than others? *Psychopharmacology (Berl.)*, 148: 3–15.
- Remington, G., Saha, A., Chong, S.A. and Shammi, C. (2005) Augmentation strategies in treatment-resistant schizophrenia. *CNS Drugs*, 19: 843–872.
- Reyntjens, A., Gelders, Y., Hoppenbrouwers, M.L. and Bussche, G.V. (1986) Thymosthenic effects of ritanserin (R 5567), a centrally acting serotonin-S₂ receptor blocker. *Drug Dev. Res.*, 8: 205–211.
- Robinson, D.G., Woerner, M.G., McMeniman, M., Mendelowitz, A. and Bilder, R.M. (2004) Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. *Am. J. Psychiatry*, 161: 473–479.
- Rollema, H., Lu, Y., Schmidt, A.W., Sprouse, J.S. and Zorn, S.H. (2000) 5-HT_{1A} receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biol. Psychiatry*, 48: 229–237.
- Rollema, H., Lu, Y., Schmidt, A.W. and Zorn, S.H. (1997) Clozapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. *Eur. J. Pharmacol.*, 338: R3–R5.
- Roth, B.L. and Meltzer, H.Y. (1995) The role of serotonin in schizophrenia. In: Bloom F.E. and Kupfer D. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, Ltd., New York, NY, pp. 1215–1227.
- Saeedi, H., Remington, G. and Christensen, B.K. (2006) Impact of haloperidol, a dopamine D₂ antagonist, on cognition and mood. *Schizophr. Res.*, 85: 222–231.
- Sams-Dodd, F. (1996) Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. *Behav. Pharmacol.*, 7: 3–23.
- Sarhan, H., Cloez-Tayarani, I., Massot, O., Fillion, M.P. and Fillion, G. (1999) 5-HT_{1B} receptors modulate release of [³H]dopamine from rat striatal synaptosomes. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 359: 40–47.
- Savina, I. and Beninger, R.J. (2007) Schizophrenic patients treated with clozapine or olanzapine perform better on theory of mind tasks than those treated with risperidone or typical antipsychotic medications. *Schizophr. Res.*, 94: 128–138.
- Schmidt, C.J., Sorensen, S.M., Kehne, J.H., Carr, A.A. and Palfreyman, M.G. (1995) The role of 5-HT_{2A} receptors in antipsychotic activity. *Life Sci.*, 56: 2209–2222.
- Schmidt, C.J., Sullivan, C.K. and Fadaye, G.M. (1994) Blockade of striatal 5-hydroxytryptamine₂ receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.*, 62: 1382–1389.
- Schultz, W., Apicella, P. and Ljungberg, T. (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J. Neurosci.*, 13: 900–913.
- Seeman, P. (2002) Atypical antipsychotics: mechanism of action. *Can. J. Psychiatry*, 47: 27–38.
- Seeman, P. (2006) Dopamine receptors, schizophrenia and antipsychotics. *Can. J. Diagn.*, (January): 1–7.
- Seeman, P., Lee, T., Chau-Wong, M. and Wong, K. (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature*, 261: 717–719.
- Seeman, P., Schwarz, J., Chen, J.F., Szechtman, H., Perreault, M., McKnight, G.S., Roder, J.C., Quirion, R., Boksa, P., Srivastava, L.K., Yanai, K., Weinshenker, D. and Sumiyoshi, T. (2006) Psychosis pathways converge via D_{2high} dopamine receptors. *Synapse*, 60: 319–346.
- Seeman, P. and Talerico, T. (1998) Antipsychotic drugs which elicit little or no parkinsonism bind more loosely than dopamine to brain D₂ receptors, yet occupy high levels of these receptors. *Mol. Psychiatry*, 3: 123–134.
- Seeman, P. and Talerico, T. (1999) Rapid release of antipsychotic drugs from dopamine D₂ receptors: an explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. *Am. J. Psychiatry*, 156: 876–884.
- Sepehry, A.A., Potvin, S., Elie, R. and Stip, E. (2007) Selective serotonin reuptake inhibitor (SSRI) add-on therapy for the negative symptoms of schizophrenia: a meta-analysis. *J. Clin. Psychiatry*, 68: 604–610.
- Sergi, M.J., Green, M.F., Widmark, C., Reist, C., Erhart, S., Braff, D.L., Kee, K.S., Marder, S.R. and Mintz, J. (2007a) Social cognition and neurocognition: effects of risperidone, olanzapine, and haloperidol. *Am. J. Psychiatry*, 164: 1585–1592.
- Sergi, M.J., Rassovsky, Y., Widmark, C., Reist, C., Erhart, S., Braff, D.L., Marder, S.R. and Green, M.F. (2007b) Social cognition in schizophrenia: relationships with neurocognition and negative symptoms. *Schizophr. Res.*, 90: 316–324.
- Siuciak, J.A., Chapin, D.S., McCarthy, S.A., Guanowsky, V., Brown, J., Chiang, P., Marala, R., Patterson, T., Seymour, P.A., Swick, A. and Iredale, P.A. (2007) CP-809,101, a selective 5-HT_{2C} agonist, shows activity in animal models of antipsychotic activity. *Neuropharmacology*, 52: 279–290.
- Sokoloff, P., Diaz, J., Levesque, D., Pilon, C., Dimitriadou, V., Griffon, N., Lammers, C.H., Martres, M.P. and Schwartz, J.C. (1995) Novel dopamine receptor subtypes as targets for antipsychotic drugs. *Ann. N. Y. Acad. Sci.*, 757: 278–292.
- Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.L. and Schwartz, J.C. (1990) Molecular cloning and characterization of a novel dopamine receptor D₃ as a target for neuroleptics. *Nature*, 347: 146–151.
- Stephenson, C.M., Bigliani, V., Jones, H.M., Mulligan, R.S., Acton, P.D., Visvikis, D., Ell, P.J., Kerwin, R.W. and Pilowsky, L.S. (2000) Striatal and extra-striatal D₂/D₃ dopamine receptor occupancy by quetiapine in vivo. ^[(123)I]-epidepride single photon emission tomography (SPET) study. *Br. J. Psychiatry*, 177: 408–415.
- Su, Y.A., Si, T.M., Zhou, D.F., Guo, C.M., Wang, X.D., Yang, Y., Shu, L. and Liang, J.H. (2007) Risperidone attenuates MK-801-induced hyperlocomotion in mice via the blockade

- of serotonin 5-HT_{2A/2C} receptors. *Eur. J. Pharmacol.*, 564: 123–130.
- Sullivan, P.F. (2008) Schizophrenia genetics: the search for a hard lead. *Curr. Opin. Psychiatry*, 21: 157–160.
- Tamminga, C.A. (2002) Partial dopamine agonists in the treatment of psychosis. *J. Neural Transm.*, 109: 411–420.
- Tanda, G., Frau, R. and Di Chiara, G. (1995) Local 5HT₃ receptors mediate fluoxetine but not desipramine-induced increase of extracellular dopamine in the prefrontal cortex. *Psychopharmacology (Berl.)*, 119: 15–19.
- Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F. and Julius, D. (1995) Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors. *Nature*, 374: 542–546.
- Tiihonen, J., Kuoppamäki, M., Nagren, K., Bergman, J., Eronen, E., Syvälahti, E. and Hietala, J. (1996) Serotonergic modulation of striatal D₂ dopamine receptor binding in humans measured with positron emission tomography. *Psychopharmacology (Berl.)*, 126: 277–280.
- van den Buuse, M., Garner, B., Gogos, A. and Kusljic, S. (2005) Importance of animal models in schizophrenia research. *Aust. N. Z. J. Psychiatry*, 39: 550–557.
- van den Buuse, M. and Gogos, A. (2007) Differential effects of antipsychotic drugs on serotonin_{1A} receptor-mediated disruption of prepulse inhibition. *J. Pharmacol. Exp. Ther.*, 320: 1224–1236.
- Van Rossum, J. (1967) The significance of dopamine-receptor blockade for the action of neuroleptic drugs. In: Coyle J., Brill H., Deniker P., Hippius H. and Bradley P.B. (Eds.), *Neuropsychopharmacology, Proceedings of the 5th Colloquium Internationale Neuro-psychopharmacologicum (CINP)*. Excerpta Medica, Amsterdam, pp. 321–329.
- Verdoux, H. and Cougnard, A. (2006) Schizophrenia: who is at risk? Who is a case? *Int. Clin. Psychopharmacol.*, 21(Suppl. 2): S17–S19.
- Verhoeff, N.P., Christensen, B.K., Hussey, D., Lee, M., Papatheodorou, G., Kopala, L., Rui, Q., Zipursky, R.B. and Kapur, S. (2003) Effects of catecholamine depletion on D₂ receptor binding, mood, and attentiveness in humans: a replication study. *Pharmacol. Biochem. Behav.*, 74: 425–432.
- Vollenweider, F.X., Vontobel, P., Hell, D. and Leenders, K. (1999) 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man – a PET study with [¹¹C]raclopride. *Neuropsychopharmacology*, 20: 424–433.
- Voruganti, L., Slomka, P., Zabel, P., Costa, G., So, A., Mattar, A. and Awad, A.G. (2001) Subjective effects of AMPT-induced dopamine depletion in schizophrenia: correlation between dysphoric responses and striatal D₂ binding ratios on SPECT imaging. *Neuropsychopharmacology*, 25: 642–650.
- Voruganti, L.N. and Awad, A.G. (2006) Subjective and behavioural consequences of striatal dopamine depletion in schizophrenia — findings from an in vivo SPECT study. *Schizophr. Res.*, 88: 179–186.
- Waddington, J.L., Lane, A., Scully, P.J., Larkin, C. and O'Callaghan, E. (1998) Neurodevelopmental and neuroprogressive processes in schizophrenia. Antithetical or complementary, over a lifetime trajectory of disease? *Psychiatr. Clin. N. Am.*, 21: 123–149.
- Wadenberg, M.L. (1992) Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. *J. Neural Transm. Gen. Sect.*, 89: 49–59.
- Waldmeier, P.C. and Delini-Stula, A.A. (1979) Serotonin-dopamine interactions in the nigrostriatal system. *Eur. J. Pharmacol.*, 55: 363–373.
- Walker, E. (2002) Risk factors and the neurodevelopmental course of schizophrenia. *Eur. Psychiatry*, 17: 363–369.
- Weinberger, D.R. (1987) Implications of normal brain development for the pathogenesis of schizophrenia. *Arch. Gen. Psychiatry*, 44: 660–669.
- Weinberger, D.R., Berman, K.F. and Illowsky, B.P. (1988) Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. III. A new cohort and evidence for a monoaminergic mechanism. *Arch. Gen. Psychiatry*, 45: 609–615.
- Wong, A.H. and Van Tol, H.H. (2003) The dopamine D₄ receptors and mechanisms of antipsychotic atypicality. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1091–1099.
- Woolley, D.W. and Shaw, E. (1954) A biochemical and pharmacological suggestion about certain mental disorders. *Proc. Natl. Acad. Sci. U.S.A.*, 40: 228–231.
- Xiberas, X., Martinot, J.L., Mallet, L., Artiges, E., Canal, M., Loc'h, C., Maziere, B. and Paillere-Martinot, M.L. (2001a) In vivo extrastriatal and striatal D₂ dopamine receptor blockade by amisulpride in schizophrenia. *J. Clin. Psychopharmacol.*, 21: 207–214.
- Xiberas, X., Martinot, J.L., Mallet, L., Artiges, E., Loc, H.C., Maziere, B. and Paillere-Martinot, M.L. (2001b) Extrastriatal and striatal D₂ dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. *Br. J. Psychiatry*, 179: 503–508.
- Yamamoto, B.K., Nash, J.F. and Gudelsky, G.A. (1995) Modulation of methylenedioxymethamphetamine-induced striatal dopamine release by the interaction between serotonin and gamma-aminobutyric acid in the substantia nigra. *J. Pharmacol. Exp. Ther.*, 273: 1063–1070.
- Zazpe, A., Artaiz, I., Innerarity, A., Del Olmo, E., Castro, E., Labeaga, L., Pazos, A. and Orjales, A. (2006) In vitro and in vivo characterization of F-97013-GD, a partial 5-HT_{1A} agonist with antipsychotic- and antiparkinsonian-like properties. *Neuropharmacology*, 51: 129–140.
- Zhang, W., Perry, K.W., Wong, D.T., Potts, B.D., Bao, J., Tollefson, G.D. and Bymaster, F.P. (2000) Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology*, 23: 250–262.

CHAPTER 7

Serotonin and dopamine interactions in psychosis prevention

Neil M. Richtand^{1,2,*} and Robert K. McNamara²

¹Cincinnati Veterans Affairs Medical Center, Psychiatry Service (V116A), 3200 Vine Street, Cincinnati, OH 45220, USA

²Department of Psychiatry, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA

Abstract: There has been significant recent growth in programmes evaluating preventive treatment for individuals exhibiting prodromal symptoms, at high risk of developing first-episode psychosis. Because of the tremendous human and economic burden of schizophrenia and other psychotic disorders, primary prevention modalities of even modest impact would likely have important public health consequence. Several published clinical trials suggest that antipsychotic medications have beneficial effects in either preventing or postponing the emergence of first-episode psychosis in individuals at high risk of psychosis. It is not clear, however, that antipsychotic drugs are the most effective, or safest, pharmacological treatment for psychosis prevention. Mechanisms for primary prevention (intervening to remove a cause of illness) and treatment are not necessarily similar. All of the medications developed for treatment of psychosis rely on tertiary prevention, and there is no a priori reason to assume that these treatments would be the safest and most effective primary preventive treatment of first-episode psychosis. Evidence suggests that selective serotonin reuptake inhibitors, serotonin 5-HT_{2A} and dopamine D₃ receptor antagonists, mood-stabilizing medications, GABAergic, glutamatergic and neuroprotective compounds may also be beneficial primary prevention drugs for first-episode psychosis. While there are indications that effective preventive interventions are feasible, data on safety and efficacy of primary preventive treatment interventions are limited and published studies highlight the enrolment challenges facing efforts to identify the safest and most effective preventive treatment interventions through human clinical trials. Treatments preventing behavioural alterations using developmental animal models with relevance to limbic system neurobiology could therefore be useful in focusing hypotheses regarding effective treatments for psychosis prevention. In one such study, low-dose risperidone pre-treatment prevented behavioural abnormalities following neonatal hippocampal lesions, while higher risperidone pre-treatment was ineffective. These findings support the predictive validity of the neonatal hippocampal lesion model in identifying psychosis prevention interventions, provide theoretical support for the use of low-dose risperidone in prevention of first-episode psychosis and suggest the possibility that higher risperidone doses could be less effective than low dosages in this application. These observations also suggest a potential role for selective 5-HT_{2A} receptor antagonists as drug development targets for psychosis prevention.

Keywords: dopamine; serotonin; schizophrenia; psychosis; neuroleptic; antipsychotic; prodrome; prodromal; prevention

*Corresponding author. Tel.: +1 513 558 6657;
Fax: +1 513 558 0042; E-mail: neil.richtand@uc.edu

Introduction

Preventive interventions in medicine can be divided into primary, secondary and tertiary preventions (Fletcher et al., 1966). Primary prevention eliminates disease occurrence by removing a disease-causing mechanism, such as use of statins to lower cholesterol and prevent myocardial infarction. Secondary prevention detects disease at an early stage of illness, prior to symptomatic problems, such as screening colonoscopies and mammograms. Tertiary prevention intervenes after the disease has become manifest, preventing further disease progression. All of the medications developed for treatment of psychosis rely on tertiary prevention, and there is no a priori reason to assume that these treatments would be the safest and most effective primary preventive treatment of first-episode psychosis. Because of the tremendous human and economic burden of schizophrenia and other psychotic disorders (Rice, 1999), however, primary prevention modalities of even modest impact would likely have significant public health consequence.

Outcome in psychosis prevention studies

Is psychosis in schizophrenia preventable? Such an intervention, if possible, could dramatically improve treatment of this severe, debilitating and typically lifelong condition. There has been tremendous recent growth in programmes evaluating preventive treatment for individuals at high risk of developing first-episode psychosis (Cornblatt, 2002; McGorry et al., 2002; Woods et al., 2003; McGlashan et al., 2006; Addington et al., 2007). Programmes have been initiated in a number of centres to characterize the pre-illness stage of the pre-psychotic prodrome, identify and characterize genetic and environmental vulnerability factors, address ethical issues inherent in early intervention strategies, and attempt to identify effective primary prevention treatments (Cornblatt et al., 2001; McGorry et al., 2001). Work by Cornblatt and colleagues, characterizing treatment interventions in a naturalistic setting, suggests antidepressant and antipsychotic medications are equally effective in improving clinical outcome (Cornblatt,

2002; Cornblatt et al., 2002). While conclusions from these findings are limited because the data were not collected in a randomized, placebo-controlled, blinded study design, it is nonetheless an important observation challenging the conventional viewpoint that antipsychotic medications should be the first line of pharmacotherapy for preventive treatment. A subsequent prospective, naturalistic study of 48 adolescents in the prodromal, pre-psychotic phase of schizophrenia compared the effectiveness of pharmacological intervention with antidepressant ($N = 20$) vs. second-generation antipsychotic ($N = 28$) treatments in preventing conversion to psychosis (Cornblatt et al., 2007). Patients receiving antipsychotic monotherapy ($N = 12$) and antipsychotic plus antidepressant medication ($N = 16$) were both assigned to the antipsychotic treatment group, while patients receiving antidepressant medication, alone or in combination with medications other than antipsychotics, were assigned to the antidepressant treatment group. A striking difference in time to conversion to psychosis was observed between treatment groups, with patients in the antidepressant medication group significantly less likely to convert to psychosis compared to patients in the antipsychotic medication treatment group (Kaplan–Meier log-rank test $p = .007$) (Cornblatt et al., 2007). While conclusions from these observations are also limited because the data were not collected in a randomized or blinded study design and because the preponderance of medications used in the antidepressant treatment group included serotonin (5-HT) reuptake inhibition as a mechanism of action, these observations also suggest the mechanistic possibility that 5-HT reuptake inhibition may be an effective medication development target for psychosis prevention.

In contrast to the observations described above, a randomized trial in individuals at high risk for progression to first-episode psychosis compared low-dose risperidone (average dose 1.3 mg/day) plus cognitive behaviour therapy to treatment as usual (case management, supportive psychotherapy and as-needed pharmacotherapy which could include antidepressant or benzodiazepine, but not antipsychotic medications). Differences in progression to first-episode psychosis between treatment groups

suggest that low-dose risperidone significantly reduced the incidence of first-episode psychosis (McGorry et al., 2002). In that study, from among 522 individuals identified as at risk for conversion to psychosis, 92 subjects agreed to participate in a research study, 59 were randomized to treatment and 14 were fully adherent with the pharmacological intervention. These numbers highlight the extreme challenges facing human research in this field. These enrolment challenges are further highlighted by the findings of a third study, a double-blind, randomized, placebo-controlled study of olanzapine (5–15 mg/day) in subjects experiencing prodromal symptoms. In that study, 16% of olanzapine patients vs. 38% of placebo patients converted to psychosis during a one-year treatment period. This difference did not reach statistical significance, however, likely due to limited study enrolment (olanzapine treatment group $N = 31$; placebo treatment group $N = 29$). During a one-year follow-up period in which both groups received no medication, the conversion rate to psychosis was similar in both groups (McGlashan et al., 2006). In summary, while an overwhelming clinical need mandates treatment in many cases for individuals in the prodromal stages of a pre-psychotic illness, and while there are indications that effective preventive interventions are feasible, data on safety and efficacy of primary preventive treatment interventions are limited. Published studies highlight the enrolment challenges facing efforts to identify the safest and most effective preventive treatment interventions through human clinical trials.

New directions in psychosis prevention

Several major impediments stand in the way of research efforts to identify effective primary prevention treatments for first-episode psychosis. First, the most effective primary prevention interventions would target the aetiology of the illness. At present, the aetiology(s) of schizophrenia and other psychotic disorders are not known, necessitating screening of many diverse compounds to identify the most effective candidates for primary prevention. For example, the current understanding of the pathophysiology of psychotic

illness suggests that GABAergic, glutamatergic and/or neuroprotective compounds would be potential pharmacological targets for psychosis prevention medications (Wassef et al., 1999; Carlsson et al., 2003; Casey et al., 2003; Tsai and Coyle, 2003). Similar arguments, however, could also be made for 5-HT_{2A} receptor antagonists (Lieberman et al., 1998; Roth et al., 2004; Richtand et al., 2006), or antidepressant and mood-stabilizing medications (Post et al., 1982; Cornblatt, 2002; Cornblatt et al., 2002, 2007). We and others have also presented data suggesting that D3 receptor antagonists would be an effective medication class for primary prevention against psychotic disorders (Henry et al., 1995; Flores et al., 1996a; Wallace et al., 1996; Wan and Corbett, 1997; Richtand et al., 2000, 2001b, 2003; Chiang et al., 2003; Richtand, 2006; Vogel et al., 2006). While it is therefore likely that it will be necessary to test a diverse array of chemical classes to identify an effective primary prevention treatment for first-episode psychosis, in practice, the risk to human subjects, as well as the practical limitations of limited clinical trial enrolment, makes it exceedingly difficult to screen even a small number of compounds in human subjects. Second, for any drug holding clinical promise for use in primary prevention, dose–response data are needed. Again, limited subject enrolment in primary prevention clinical trials, and the risks of study participation, impedes obtaining this critically needed information in human subjects. These observations suggest a significant need for animal models with predictive validity in the first-episode psychosis prevention field. Information gained from animal models could significantly overcome these obstacles by identifying a smaller subset of compounds with greatest potential for study in human clinical trials.

Pharmacological role of serotonin and dopamine in psychosis prevention

Serotonin receptors

5-HT receptors are divided among seven families. Six of the families code for G-protein-coupled receptors, and one family (5-HT₃) codes for a

5-HT-gated ion channel receptor (Raymond et al., 2001). The 5-HT₂ receptor family is further subdivided among the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptor subtypes. Interest in the potential role of 5-HT₂ receptors in psychosis has been stimulated by the observation that atypical antipsychotic medications share high affinity binding to 5-HT_{2A} and 5-HT_{2C} receptors (Meltzer et al., 1989). For example, it has been suggested that the therapeutic effect of risperidone may be mediated by a combination of high affinity 5-HT_{2A} receptor antagonism and partial dopamine D2 receptor occupancy, and that high D2 dopamine receptor occupancy is unnecessary for risperidone's antipsychotic effect (Leysen et al., 1993; Schotte et al., 1996).

5-HT_{2A} receptor distribution and function

5-HT exerts its effects through a widely distributed network of neurons. Serotonergic projections are distributed widely throughout the brain from 5-HT cell bodies located in the dorsal and ventral raphe nuclei. The cerebral cortex receives a particularly dense distribution of innervation of serotonergic neurons, and 5-HT_{2A} receptors play an important role in modulation of neuronal circuitry in both the medial prefrontal cortex and the hippocampus (Harvey, 2003). In prefrontal cortex, 5-HT_{2A} receptors are expressed postsynaptically on pyramidal cells and local circuit neurons (Xia et al., 2003). In the ventral tegmentum, 5-HT_{2A} receptors are expressed on the cell bodies of dopamine neurons, and play an important role in modulating dopamine neuronal function (Doherty and Pickel, 2000; Nocjar et al., 2002). Serotonergic systems interact with dopaminergic systems at a variety of different anatomical levels, and modulation of 5-HT receptor function can have differential effects on dopamine neuronal activity (Lieberman et al., 1998). Electrophysiologically, 5-HT inhibition of dopamine neuronal firing is blocked by 5-HT_{2A} receptor antagonists (Ugedo et al., 1989). 5-HT_{2A} antagonists also reduce the number of spontaneously active dopaminergic neurons in the ventral tegmentum (Chiodo and Bunney, 1983; Sorensen et al., 1993). Overall, 5-HT_{2A} receptor antagonists generally block dopamine-mediated behaviours (Lieberman et al., 1998). It has been suggested

that contradictory data regarding the functional role of 5-HT_{2A} receptor antagonists in the interaction between serotonergic and dopaminergic systems may be accounted for by a model in which 5-HT_{2A} receptor antagonists functionally stabilize dopaminergic neurotransmission (Roth et al., 2004).

5-HT_{2A} receptor involvement in pre-psychotic cognitive processes

One of the earliest links between 5-HT_{2A} receptors and psychosis was the observation that the hallucinogenic properties of LSD result from the 5-HT_{2A} agonist properties of that compound (Glennon et al., 1984). More recent interest in 5-HT_{2A} receptor involvement in psychosis has expanded to include the role of the 5-HT_{2A} receptor in attentional and cognitive deficits which may serve as a pre-psychotic foundation for fragmentation into psychotic thinking. Neurocognitive and information-processing deficits identified as vulnerability markers for schizophrenia have face validity based on patient reports of difficulties with concentration, memory and filtering extraneous sensory information (Braff and Geyer, 1990), and several information-processing and cognition measures have been evaluated as vulnerability markers for schizophrenia (Cornblatt and Malhotra, 2001; Braff and Freedman, 2002; Gottesman and Gould, 2003). Among these vulnerability factors, there is strong evidence demonstrating an important role for the 5-HT_{2A} receptor in deficits in pre-pulse inhibition of the startle response (Bakshi et al., 1994; Varty et al., 1999), an operational measure of sensorimotor gating which is impaired in schizophrenia patients and their first-degree unaffected relatives (Braff et al., 1992; Cadenhead et al., 2000). Evidence also suggests 5-HT_{2A} receptor involvement in the neurocognitive deficits of schizophrenia (Meltzer et al., 2003; Roth et al., 2004).

Summary

5-HT_{2A} receptors play an important role in attentional, information-processing and cognitive

functions which may be trait markers for psychosis vulnerability. The involvement of the 5-HT_{2A} receptor in functions related to psychosis vulnerability suggests a potential role for highly selective 5-HT_{2A} receptor antagonists as drug development targets for psychosis prevention.

Dopamine receptors as pharmacological targets for psychosis prevention

The five dopamine receptor subtypes (D1–D5) are members of the superfamily of G-protein-coupled receptors and are divided between two families differing in biochemical and pharmacological properties (Spano et al., 1978). In vitro D1-family receptors (D1 and D5) couple to G_s stimulatory proteins, activating adenylyl cyclase, while D2-family receptors (D2, D3 and D4) couple to G_i inhibitory proteins, inhibiting adenylyl cyclase. Dopamine systems exert functional effects on more rapidly acting ionotropic glutamatergic, GABAergic and nicotinic cholinergic neuronal systems via modulation of the activity of second messenger signalling pathways (Strange, 1988).

D3 dopamine receptor

D3 dopamine receptor mRNA and protein are expressed primarily in phylogenetically ancient limbic brain regions linked to motivated and emotional behaviours, including olfactory tubercle, nucleus accumbens, islands of Calleja (located ventral to the ventral pallidum and nucleus accumbens), substantia nigra and ventral tegmentum, and also within prefrontal cortex (Bouthenet et al., 1991; Levesque et al., 1992; Landwehrmeyer et al., 1993; Richtand et al., 1995; Khan et al., 1998; Levant, 1998; Gurevich and Joyce, 1999; Diaz et al., 2000). The earliest reports describing the highly restricted expression pattern of the D3 receptor suggested a role for this receptor in psychosis (Sokoloff et al., 1990, 1992). The cellular pattern of D3 protein expression does not overlap with expression of synaptic proteins such as synaptophysin, suggesting that receptor localization is primarily extrasynaptic (Diaz et al., 2000). Protein and mRNA expression are highly co-localized, suggesting receptor expression occurs

primarily on perikarya, proximal dendrites and short axons as opposed to long axon terminals from other brain regions (Levesque et al., 1992). D3 receptor protein has been described in tyrosine hydroxylase-positive neurons in substantia nigra and ventral tegmentum, indicative of presynaptic D3 receptors (Diaz et al., 2000), although the functional role of these D3 autoreceptors has not yet been fully elucidated (Koeltzow et al., 1998; L'hirondel et al., 1998).

D3 receptor function is of particular interest as a potential pharmacological target for psychosis prevention interventions because evidence suggests its effects are primarily inhibitory (Piercey et al., 1992; Waters et al., 1993; Griffon et al., 1995; Accili et al., 1996; Flores et al., 1996a; Sigala et al., 1997; Xu et al., 1997; Duaux et al., 1998; Ekman et al., 1998; Menalled et al., 1999; Betancur et al., 2001; Pritchard et al., 2003), and that loss of this inhibitory function might contribute pathologically to neuropsychiatric disease (Flores et al., 1996a; Richtand et al., 2001a, b). The D3 receptor has highest dopamine affinity, and is the only dopamine receptor subtype occupied in the range of basal dopamine concentrations. D3 dopamine receptor blockade would be effective in preventing development of psychosis because, according to the 'D3 dopamine receptor hypothesis', stress or other environmental factors elevate basal dopamine concentrations prior to the development of psychosis. The resulting persistent D3 receptor stimulation leads to a subsequent compensatory down-regulation of D3 receptor function, and release of limbically modulated behaviours from D3 receptor-mediated inhibition (Richtand et al., 2001b, 2003, 2005; Richtand, 2006). For example, the D3 receptor inhibits novelty-stimulated locomotion (Accili et al., 1996; Xu et al., 1997; Ekman et al., 1998; Menalled et al., 1999; Pritchard et al., 2003) and amphetamine-stimulated locomotion (Waters et al., 1993) in rodents, and may also inhibit expression of analogous limbically modulated behaviours in humans including paranoia, delusions and hallucinations. The loss of D3 receptor-mediated 'brake' on these behaviours would be expressed as the emergence of augmented locomotor response to novelty and amphetamine in rodents, and paranoid psychotic symptoms in humans.

Evidence supporting this hypothesis has been described in rodent behavioural sensitization and other ‘animal models’ of psychosis (Henry et al., 1995; Flores et al., 1996a; Wallace et al., 1996; Wan and Corbett, 1997; Richtand et al., 2000, 2003; Chiang et al., 2003; Richtand, 2006). For example, directly supporting the hypothesis that tolerance of D3 receptor-mediated inhibition contributes to behavioural sensitization, locomotor sensitization is inhibited if D3 receptor tolerance is prevented by pre-treatment with nafadotride, a D3 receptor antagonist (Richtand et al., 2000). Furthermore, D3 receptor mRNA and protein expression are decreased (Chiang et al., 2003), and D3 receptor function is attenuated in amphetamine-sensitized rats (Richtand et al., 2003). Decreased D3 receptor binding has also been observed in nucleus accumbens following locomotor sensitization to cocaine (Wallace et al., 1996). Further supporting the ‘D3 receptor hypothesis’, following neonatal hippocampal lesion, a developmental model of abnormal behaviour with relevance to schizophrenia (Lipska and Weinberger, 2000) (described in more detail below), dopamine D3 receptor binding is decreased following lesion (Flores et al., 1996a), while locomotor response to D2 agonists is increased, suggesting that hyperlocomotor behavioural responses result from a decrease in inhibitory D3 receptor function (Wan and Corbett, 1997). Lending additional support to this general model of augmented locomotor responsiveness, D3 receptor binding is also decreased in nucleus accumbens in rat offspring exposed to stress in late pregnancy, a manipulation which also results in increased locomotor response to novelty, more rapid locomotor sensitization and increased amphetamine self-administration (Henry et al., 1995).

Summary

Several plausible targets for psychosis prevention involving serotonergic and dopaminergic neurotransmission have been identified. These targets may play a developmental role, modulating limbic system activity in adaptation to maturational factors impacting vulnerability to psychosis such as altered sex hormone levels and exposure to

stress or drugs of abuse. Elucidating the roles of 5-HT and dopamine in limbic system maturation may help identify specific mechanisms which could be exploited for psychosis prevention. Studies using developmental animal models with the potential to elucidate the roles of 5-HT and dopamine in limbic system development may be particularly useful for this purpose.

Animal developmental models and psychosis prevention

Several rodent developmental models have been identified in which abnormalities in limbically modulated behaviours emerge following puberty, providing the opportunity to characterize both the normal and the abnormal development of circuits with relevance to psychosis (Lipska et al., 1993; Lipska and Weinberger, 2000; Shi et al., 2003; Zuckerman et al., 2003; Meyer et al., 2005; Moore et al., 2006). One of the earliest and most thoroughly characterized of these developmental models is the neonatal hippocampal lesion model developed by Lipska and Weinberger. Longstanding behavioural abnormalities, including increased locomotor responsiveness to stress, novel environment and amphetamine (Lipska et al., 1993), and deficits in pre-pulse inhibition of startle (Lipska et al., 1995; Swerdlow et al., 1995) emerge after puberty in rats following neonatal hippocampal lesion. Neonatal hippocampal lesioned rats also exhibit deficits in social behaviour (Sams-Dodd et al., 1997) and cognition (Chambers et al., 1996) both during the pre-pubertal period and in adulthood, which may be analogous to the negative and neurocognitive deficits, respectively, observed in schizophrenic patients. Because both the time course of development of abnormal behaviours and the behaviours themselves share similarities with schizophrenia, treatments inhibiting behavioural alterations using this animal model could have predictive validity in identifying compounds effective in treating a range of symptoms of schizophrenia (Lipska and Weinberger, 2000). Studies have described the effectiveness of haloperidol (Lipska et al., 1993; Lipska and Weinberger, 1994b), clozapine (Lipska and

Weinberger, 1994b; Rueter et al., 2004) and risperidone (Rueter et al., 2004) in suppressing elevated spontaneous locomotor activity in adult neonatally lesioned rats, which may be analogous to alleviating psychotic symptoms (Lipska and Weinberger, 2000). Monitoring the development and expression of abnormal limbically mediated behaviours following neonatal hippocampal lesion could therefore provide a model with predictive validity in identifying primary preventive treatments for schizophrenia and first-episode psychosis (Lipska and Weinberger, 1994b, 2000; Sams-Dodd et al., 1997; Al-Amin et al., 2000).

Pathophysiology of neonatal hippocampal lesion

Development of the neonatal hippocampal lesion model was based in part on the observation that hippocampal pathology has been among the more robust findings in schizophrenia research (Bogerts et al., 1993; Lipska et al., 1993). While the mechanism underlying altered behaviour following neonatal lesion is not known, two limbic connections of the ventral hippocampus could account for the observed behavioural disturbances. First, excitatory glutamatergic projections from ventral hippocampus to nucleus accumbens are believed to interact with dopaminergic innervation of intrinsic cells of the nucleus accumbens (Sesack and Pickel, 1990). Loss of this excitatory input to accumbens could lead directly to increased accumbens dopamine release, perhaps through an inhibitory interneuron, or alternatively to increased dopamine responsivity in accumbens. Alternatively, hippocampal lesion also disrupts projections from hippocampus to prefrontal cortex (Jay and Witter, 1991). Loss of this projection could eventually result in loss of cortical inhibition of projections from prefrontal cortex to accumbens and cortex to ventral tegmentum, thereby increasing accumbens dopamine release or dopamine responsivity. In support of this latter model, intact prefrontal cortical function is required for expression of behavioural abnormalities following neonatal hippocampal lesion (Lipska et al., 1998). While a detailed understanding of the mechanism underlying behavioural abnormalities in this model is

not known, a major advantage of the neonatal hippocampal model is that it is not theoretically tied to dopamine abnormalities, allowing testing of a wide range of mechanisms (Lipska and Weinberger, 2000).

Risperidone pre-treatment reduces hyperlocomotor responses following neonatal hippocampal lesion

In order to test whether the neonatal hippocampal lesion model has predictive validity in identifying effective treatment strategies for prevention of first-episode psychosis, we evaluated the effect of risperidone, previously studied for prevention of first-episode psychosis (McGorry et al., 2002), in preventing the appearance of abnormally elevated locomotor behaviour following neonatal lesion. Rat pups received hippocampal or sham lesions on postnatal day 7, followed by treatment with risperidone or vehicle from postnatal days 35 to 56. In the rat, postnatal days 29 to approximately 44 are the period of pre-puberty, and postnatal days ~45 to 60–70 represent the pubertal period of sexual maturation (Zicha and Kunes, 1999). Behavioural abnormalities following neonatal lesion are not apparent at postnatal day 35, but are manifest when animals are tested at postnatal day 56 (Lipska et al., 1993). Once daily risperidone or saline injections between postnatal days 35 and 56 spanned the pre-pubertal and pubertal periods encompassing the period of development of behavioural abnormalities following neonatal lesion. The risperidone dosages chosen for study (45 µg/kg and 85 µg/kg s.c.) have significant 5-HT_{2A} receptor antagonism, with partial D2 dopamine receptor occupancy which increases with ascending dose from 45 to 85 µg/kg.

On postnatal day 57, one day following the completion of treatment, all animals received a battery of behavioural tests. Three independent behavioural measures were used to evaluate differences between lesioned and sham-lesioned treatment groups, including two previously described locomotion measures, response to amphetamine injection (1.5 mg/kg subcutaneous) and response to a novel environment (Lipska et al., 1993; Lipska and Weinberger, 1994a, 1995; Flores et al., 1996a, b;

Wan et al., 1996; Swerdlow et al., 2001). Initial locomotor response to change between light and dark cycles (first 2-h 'lights-off' period on the first evening housed in a residential activity chamber) was also employed as a third measure to identify behavioural differences between treatment groups. The human behavioural response to amphetamine in normal subjects frequently includes psychotic symptoms of suspiciousness, paranoia, delusions of persecution and hallucinations. In schizophrenia patients, amphetamine induces abnormally elevated dopamine levels (Breier et al., 1997; Abi-Dargham et al., 1998), and the behavioural expression of elevated dopamine in these patients was 'transient emergence or worsening of positive symptoms' (Abi-Dargham et al., 1998), providing a compelling link between human psychotic symptoms and the rodent behavioural response to amphetamine. The rodent hyperlocomotor response to amphetamine, which evolves from exploration and examination of the environment to more complex patterns of stereotyped behaviours, therefore has both face and predictive validity as an animal model of psychosis (Lipska and Weinberger, 2000).

When tested on postnatal day 57, animals in the risperidone 45 µg/kg pre-treatment group had a significantly reduced hyperlocomotor response to both amphetamine (1.5 mg/kg subcutaneous) and change between light and dark cycles compared to the saline pre-treatment groups. In contrast, the higher dose risperidone 85 µg/kg pre-treatment condition did not significantly alter hyperlocomotor responses to amphetamine or change between light and dark cycles. Neither the risperidone 45 or 85 µg/kg pre-treatment conditions significantly altered the hyperlocomotor response to novelty following neonatal hippocampal lesion (Richtand et al., 2006). Because low risperidone dosages prevented elevated locomotor activity in some of the behavioural tasks following neonatal hippocampal lesions and were also effective in preventing or postponing the appearance of first-episode psychosis in human studies (McGorry et al., 2002), these data support the predictive validity of the hippocampal lesion model in identifying potential medication targets for prevention of first-episode psychosis. These data also provide theoretical support for the use of low-dose risperidone in

prevention of first-episode psychosis, and suggest the possibility that higher risperidone dosages could be less effective than low dosages in this application.

The finding that a low risperidone dose was effective in preventing behavioural abnormality in two independent behavioural measures, while a higher dosage was without benefit, was unexpected, and may provide insight into the pharmacological properties of effective treatment. The risperidone ED₅₀ for centrally acting 5-HT₂ antagonism is 14 µg/kg in rat (Megens et al., 1994), while the ED₅₀ for D2 antagonism is from 56 to 150 µg/kg (Megens et al., 1994). Therefore, the 45 µg/kg risperidone dose has significant 5-HT_{2A} receptor antagonism and partial D2 dopamine receptor occupancy, while at the higher 85 µg/kg dose, 5-HT_{2A} saturation is similar while binding is increased at receptor populations with lower risperidone binding affinity, including dopamine D2, D3 and D4, and 5-HT_{2C} and 5-HT_{1A} receptors. The decreased efficacy of higher risperidone dosages in preventing abnormal behaviours could therefore result from lower affinity interactions of risperidone, or its metabolite(s), with a receptor type opposing the therapeutic action of risperidone. Potential candidates for this effect include 5-HT_{1A} receptors opposing the therapeutic actions of 5-HT_{2A} receptors, as there is extensive evidence demonstrating that 5-HT_{2A} and 5-HT_{1A} receptors have opposing cellular and behavioural effects (Meltzer, 1999; Meltzer et al., 2003). An alternative explanation would be opposing actions of D3 and D2 receptors (Waters et al., 1993; Xu et al., 1997).

This finding has important limitations. Although there was a 24-h withdrawal period between the final risperidone treatment and behavioural testing, these results cannot distinguish between prevention and suppression or postponement, of emerging behavioural abnormalities following neonatal hippocampal lesion. Additional studies using later time points following treatment cessation are needed to begin to address this important issue. Because of the wealth of both well-developed animal developmental models to provide information regarding normal vs. abnormal limbic system development and the

long list of potential pharmacological interventions for psychosis prevention, further research in this field holds great promise to provide safer and more effective treatment options to patients in the prodromal stage of illness.

Abbreviations

GABA gamma-aminobutyric acid
5-HT serotonin

Acknowledgements

This work was supported by the Department of Veterans Affairs Medical Research Service (NMR) and NARSAD.

References

- Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R.M., Seibyl, J.P., Bowers, M., van Dyck, C.H., Charney, D.S., Innis, R.B. and Laruelle, M. (1998) Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiatry*, 155(6): 761–767.
- Accili, D., Fishburn, C.S., Drago, J., Steiner, H., Lachowicz, J.E., Park, B.H., Gauda, E.B., Lee, E.J., Cool, M.H., Sibley, D.R., Gerfen, C.R., Westphal, H. and Fuchs, S. (1996) A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. *Proc. Natl. Acad. Sci. U.S.A.*, 93: 1945–1949.
- Addington, J., Cadenhead, K.S., Cannon, T.D., Cornblatt, B., McGlashan, T.H., Perkins, D.O., Seidman, L.J., Tsuang, M., Walker, E.F., Woods, S.W. and Heinssen, R. (2007) North American Prodrome Longitudinal Study: a collaborative multisite approach to prodromal schizophrenia research. *Schizophr. Bull.*, 33(3): 665–672.
- Al-Amin, H.A., Weinberger, D.R. and Lipska, B.K. (2000) Exaggerated MK-801-induced motor hyperactivity in rats with the neonatal lesion of the ventral hippocampus. *Behav. Pharmacol.*, 11: 269–278.
- Bakshi, V.P., Swerdlow, N.R. and Geyer, M.A. (1994) Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J. Pharmacol. Exp. Ther.*, 271: 787–794.
- Betancur, C., Lepee-Lorgeoux, I., Cazillis, M., Accili, D., Fuchs, S. and Rostene, W. (2001) Neurotensin gene expression and behavioral responses following administration of psychostimulants and antipsychotic drugs in dopamine D(3) receptor deficient mice. *Neuropsychopharmacology*, 24(2): 170–182.
- Bogerts, B., Lieberman, J.A., Ashtari, M., Bilder, R.M., Degreef, G., Lerner, G., Johns, C. and Masiar, S. (1993) Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. *Biol. Psychiatry*, 33(4): 236–246.
- Bouthenet, M.L., Souil, E., Martres, M.P., Sokoloff, P., Giros, B. and Schwartz, J.C. (1991) Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. *Brain Res.*, 564: 203–219.
- Braff, D.L. and Freedman, R. (2002) The Importance of Endophenotypes in Studies of the Genetics of Schizophrenia. Lippincott, Williams and Wilkins, Baltimore, MD.
- Braff, D.L. and Geyer, M.A. (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch. Gen. Psychiatry*, 47: 181–188.
- Braff, D.L., Grillon, C. and Geyer, M.A. (1992) Gating and habituation of the startle reflex in schizophrenic patients. *Arch. Gen. Psychiatry*, 49: 206–215.
- Breier, A., Su, T.P., Saunders, R., Carson, R.E., Kolachana, B.S., de Bartolomeis, A., Weinberger, D.R., Weisenfeld, N., Malhotra, A.K., Eckelman, W.C. and Pickar, D. (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc. Natl. Acad. Sci. U.S.A.*, 94(6): 2569–2574.
- Cadenhead, K.S., Swerdlow, N.R., Shafer, K.M., Diaz, M. and Braff, D.L. (2000) Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am. J. Psychiatry*, 157: 1660–1668.
- Carlsson, A., Waters, N., Holm-Waters, S., Tedroff, J., Nilsson, M. and Carlsson, M.L. (2003) Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu. Rev. Pharmacol. Toxicol.*, 41: 237–260.
- Casey, D.E., Daniel, D.G., Wassef, A.A., Tracy, K.A., Wozniak, P. and Somerville, K.W. (2003) Effect of divalproex combined with olanzapine or risperidone in patients with an acute exacerbation of schizophrenia. *Neuropsychopharmacology*, 28: 182–192.
- Chambers, R.A., Moore, J., McEvoy, J.P. and Levin, E.D. (1996) Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia. *Neuropsychopharmacology*, 15: 587–594.
- Chiang, Y.C., Chen, P.C. and Chen, J.C. (2003) D(3) dopamine receptors are down-regulated in amphetamine sensitized rats and their putative antagonists modulate the locomotor sensitization to amphetamine. *Brain Res.*, 972: 159–167.
- Chiodo, L.A. and Bunney, B.S. (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J. Neurosci.*, 3: 1607–1619.
- Cornblatt, B.A. (2002) The New York high risk project to the Hillside recognition and prevention (RAP) program. *Am. J. Med. Genet.*, 114: 956–966.
- Cornblatt, B.A., Lencz, T. and Kane, J.M. (2001) Treatment of the schizophrenia prodrome: is it presently ethical? *Schizophr. Res.*, 51: 31–38.

- Cornblatt, B., Lencz, T. and Obuchowski, M. (2002) The schizophrenia prodrome: treatment and high-risk perspectives. *Schizophr. Res.*, 54: 177–186.
- Cornblatt, B.A., Lencz, T., Smith, C.W., Olsen, R., Auther, A.M., Nakayama, E., Lesser, M.L., Tai, J.Y., Shah, M.R., Foley, C.A., Kane, J.M. and Correll, C.U. (2007) Can antidepressants be used to treat the schizophrenia prodrome? Results of a prospective, naturalistic treatment study of adolescents. *J. Clin. Psychiatry*, 68(4): 546–557.
- Cornblatt, B.A. and Malhotra, A.K. (2001) Impaired attention as an endophenotype for molecular genetic studies of schizophrenia. *Am. J. Med. Genet.*, 105: 11–15.
- Diaz, J., Pilon, C., Le Foll, B., Gros, C., Triller, A., Schwartz, J.C. and Sokoloff, P. (2000) Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. *J. Neurosci.*, 20: 8677–8684.
- Doherty, M.D. and Pickel, V.M. (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.*, 864: 176–185.
- Duaux, E., Gorwood, P., Griffon, N., Bourdel, M.C., Sautel, F., Sokoloff, P., Schwartz, J.C., Ades, J., Loo, H. and Poirier, M.F. (1998) Homozygosity at the dopamine D3 receptor gene is associated with opiate dependence. *Mol. Psychiatry*, 3: 333–336.
- Ekman, A., Nissbrandt, H., Heilig, M., Dijkstra, D. and Eriksson, E. (1998) Central administration of dopamine D3 receptor antisense to rat: effects on locomotion, dopamine release and [3H]spiperone binding. *Naunyn Schmiedeberg Arch. Pharmacol.*, 358: 342–350.
- Fletcher, R.H., Fletcher, S.W. and Wagner, E.H. (1966) *Clinical Epidemiology — The Essentials* (3rd edn.). Williams and Wilkins, Baltimore, MD.
- Flores, G., Barbeau, D., Quirion, R. and Srivastava, L.K. (1996a) Decreased binding of dopamine D3 receptors in limbic subregions after neonatal bilateral lesion of rat hippocampus. *J. Neurosci.*, 16: 2020–2026.
- Flores, G., Wood, G.K., Liang, J.J., Quirion, R. and Srivastava, L.K. (1996b) Enhanced amphetamine sensitivity and increased expression of dopamine D2 receptors in postpubertal rats after neonatal excitotoxic lesions of the medial prefrontal cortex. *J. Neurosci.*, 16: 7366–7375.
- Glennon, R.A., Titeler, M. and McKenney, J.D. (1984) Evidence for 5-HT₂ involvement in the mechanism of action of hallucinogenic agents. *Life Sci.*, 35: 2505–2511.
- Gottesman, I.I. and Gould, T.D. (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. *Am. J. Psychiatry*, 160: 636–645.
- Griffon, N., Sokoloff, P., Diaz, J., Levesque, D., Sautel, F., Schwartz, J.C., Simon, P., Costentin, J., Garrido, F. and Mann, A. (1995) The dopamine D3 receptor and schizophrenia: pharmacological, anatomical and genetic approaches. *Eur. Neuropsychopharmacol.*, 5(Suppl): 3–9.
- Gurevich, E.V. and Joyce, J.N. (1999) Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology*, 20: 60–80.
- Harvey, J.A. (2003) Role of the serotonin 5-HT_{2A} receptor in learning. *Learn. Mem.*, 10: 355–362.
- Henry, C., Guegant, G., Cador, M., Arnault, E., Arsaut, J., Le Moal, M. and Demotes-Mainard, J. (1995) Prenatal stress in rats facilitates amphetamine-induced sensitization and induces long-lasting changes in dopamine receptors in the nucleus accumbens. *Brain Res.*, 685: 179–186.
- Jay, T.M. and Witter, M.P. (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of *Phaseolus vulgaris*-leucoagglutinin. *J. Comp. Neurol.*, 313: 574–586.
- Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A. and De La Calle, A. (1998) Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. *J. Comp. Neurol.*, 402: 353–371.
- Koeltzow, T.E., Xu, M., Cooper, D.C., Hu, X.T., Tonegawa, S., Wolf, M.E. and White, F.J. (1998) Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. *J. Neurosci.*, 18: 2231–2238.
- Landwehrmeyer, B., Mengod, G. and Palacios, J.M. (1993) Differential visualization of dopamine D2 and D3 receptor sites in rat brain. A comparative study using in situ hybridization histochemistry and ligand binding autoradiography. *Eur. J. Neurosci.*, 5: 145–153.
- Levant, B. (1998) Differential distribution of D3 dopamine receptors in the brains of several mammalian species. *Brain Res.*, 800: 269–274.
- Levesque, D., Diaz, J., Pilon, C., Martres, M.P., Giros, B., Souil, E., Schott, D., Morgat, J.L., Schwartz, J.C. and Sokoloff, P. (1992) Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin. *Proc. Natl. Acad. Sci. U.S.A.*, 89: 8155–8159.
- Leyens, J.E., Janssen, P.M., Schotte, A., Luyten, W.H. and Megens, A.A. (1993) Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT₂ receptors. *Psychopharmacology (Berl.)*, 112: S40–S54.
- L'hirondel, M., Cheramy, A., Godeheu, G., Artaud, F., Saiardi, A., Borrelli, E. and Glowinski, J. (1998) Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice. *Brain Res.*, 792: 253–262.
- Lieberman, J.A., Mailman, R.B., Duncan, G., Sikich, L., Chakos, M., Nichols, D.E. and Kraus, J.E. (1998) Serotonergic basis of antipsychotic drug effects in schizophrenia. *Biol. Psychiatry*, 44: 1099–1117.
- Lipska, B.K., Al-Amin, H.A. and Weinberger, D.R. (1998) Excitotoxic lesions of the rat medial prefrontal cortex. Effects on abnormal behaviors associated with neonatal hippocampal damage. *Neuropsychopharmacology*, 19: 451–464.
- Lipska, B.K., Jaskiw, G.E. and Weinberger, D.R. (1993) Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal

- damage: a potential animal model of schizophrenia. *Neuropsychopharmacology*, 9(1): 67–75.
- Lipska, B.K., Swerdlow, N.R., Geyer, M.A., Jaskiw, G.E., Braff, D.L. and Weinberger, D.R. (1995) Neonatal excitotoxic hippocampal damage in rats causes post-pubertal changes in prepulse inhibition of startle and its disruption by apomorphine. *Psychopharmacology* (Berl.), 122: 35–43.
- Lipska, B.K. and Weinberger, D.R. (1994a) Gonadectomy does not prevent novelty or drug-induced motor hyperresponsiveness in rats with neonatal hippocampal damage. *Brain Res. Dev. Brain Res.*, 78: 253–258.
- Lipska, B.K. and Weinberger, D.R. (1994b) Subchronic treatment with haloperidol and clozapine in rats with neonatal excitotoxic hippocampal damage. *Neuropsychopharmacology*, 10: 199–205.
- Lipska, B.K. and Weinberger, D.R. (1995) Genetic variation in vulnerability to the behavioral effects of neonatal hippocampal damage in rats. *Proc. Natl. Acad. Sci. U.S.A.*, 92: 8906–8910.
- Lipska, B.K. and Weinberger, D.R. (2000) To model a psychiatric disorder in animals: schizophrenia as a reality test. *Neuropsychopharmacology*, 23: 223–239.
- McGlashan, T.H., Zipursky, R.B., Perkins, D., Addington, J., Miller, T., Woods, S.W., Hawkins, K.A., Hoffman, R.E., Preda, A., Epstein, I., Addington, D., Lindborg, S., Trzaskoma, Q., Tohen, M. and Breier, A. (2006) Randomized, double-blind trial of olanzapine versus placebo in patients prodromally symptomatic for psychosis. *Am. J. Psychiatry*, 163: 790–799.
- McGorry, P.D., Yung, A. and Phillips, L. (2001) Ethics and early intervention in psychosis: keeping up the pace and staying in step. *Schizophr. Res.*, 51: 17–29.
- McGorry, P.D., Yung, A.R., Phillips, L.J., Yuen, H.P., Francey, S., Cosgrave, E.M., Germano, D., Bravin, J., McDonald, T., Blair, A., Adlard, S. and Jackson, H. (2002) Randomized controlled trial of interventions designed to reduce the risk of progression to first-episode psychosis in a clinical sample with subthreshold symptoms. *Arch. Gen. Psychiatry*, 59: 921–928.
- Megens, A.A., Awouters, F.H., Schotte, A., Meert, T.F., Dugovic, C., Niemegeers, C.J. and Leysen, J.E. (1994) Survey on the pharmacodynamics of the new antipsychotic risperidone. *Psychopharmacology* (Berl.), 114: 9–23.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21: 106S–115S.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159–1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251: 238–246.
- Menalled, L.B., Dziewczapolski, G., Garcia, M.C., Rubinstein, M. and Gershanik, O.S. (1999) D3 receptor knockdown through antisense oligonucleotide administration supports its inhibitory role in locomotion. *Neuroreport*, 10: 3131–3136.
- Meyer, U., Feldon, J., Schedlowski, M. and Yee, B.K. (2005) Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. *Neurosci. Biobehav. Rev.*, 29: 913–947.
- Moore, H., Jentsch, J.D., Ghajarnia, M., Geyer, M.A. and Grace, A.A. (2006) A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. *Biol. Psychiatry*, 60(3): 253–264.
- Nocjar, C., Roth, B.L. and Pehek, E.A. (2002) Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, 111: 163–176.
- Piercey, M.F., Lum, J.T., Hoffmann, W.E., Carlsson, A., Ljung, E. and Svensson, K. (1992) Antagonism of cocaine's pharmacological effects by the stimulant dopaminergic antagonists, (+)-AJ76 and (+)-UH232. *Brain Res.*, 588: 217–222.
- Post, R.M., Uhde, T.W., Putnam, F.W., Ballenger, J.C. and Berrettini, W.H. (1982) Kindling and carbamazepine in affective illness. *J. Nerv. Ment. Dis.*, 170: 717–731.
- Pritchard, L.M., Logue, A.D., Hayes, S., Welge, J.A., Xu, M., Zhang, J., Berger, S.P. and Richtand, N.M. (2003) 7-OH-DPAT and PD 128907 selectively activate the D3 dopamine receptor in a novel environment. *Neuropsychopharmacology*, 28: 100–107.
- Raymond, J.R., Mukhin, Y.V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T.W., Grewal, J.S. and Garnovskaya, M.N. (2001) Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.*, 92: 179–212.
- Rice, D.P. (1999) The economic impact of schizophrenia. *J. Clin. Psychiatry*, 60(Suppl 1): 4–6.
- Richtand, N.M. (2006) Behavioral sensitization, alternative splicing, and D3 dopamine receptor-mediated inhibitory function. *Neuropsychopharmacology*, 31(11): 2368–2375.
- Richtand, N.M., Goldsmith, R.J., Nolan, J.E. and Berger, S.P. (2001a) The D3 dopamine receptor and substance dependence. *J. Addict. Dis.*, 20: 19–32.
- Richtand, N.M., Kelsoe, J.R., Segal, D.S. and Kuczenski, R. (1995) Regional quantification of D1, D2, and D3 dopamine receptor mRNA in rat brain using a ribonuclease protection assay. *Brain Res. Mol. Brain Res.*, 33: 97–103.
- Richtand, N.M., Logue, A.D., Welge, J.A., Perdue, J., Tubbs, L.J., Spitzer, R.H., Sethuraman, G. and Geraciotti, T.D. (2000) The dopamine D3 receptor antagonist nafadotride inhibits development of locomotor sensitization to amphetamine. *Brain Res.*, 867(1–2): 239–242.
- Richtand, N.M., Pritchard, L.M. and Coolen, L.M. (2005) Dopamine receptor alternative splicing. In: Schmidt W.J. and Reith M.E.A. (Eds.), *Dopamine and Glutamate in Psychiatric Disorders*. Humana Press, Totowa, NJ, pp. 45–61.
- Richtand, N.M., Taylor, B., Welge, J.A., Ahlbrand, R., Ostrander, M.M., Burr, J., Hayes, S., Coolen, L.M., Pritchard, L.M., Logue, A., Herman, J.P. and McNamara, R.K. (2006) Risperidone pretreatment prevents elevated locomotor activity following neonatal hippocampal lesions. *Neuropsychopharmacology*, 31(1): 77–89.
- Richtand, N.M., Welge, J.A., Levant, B., Logue, A.D., Hayes, S., Pritchard, L.M., Geraciotti, T.D., Coolen, L.M. and Berger, S.P. (2003) Altered behavioral response to dopamine

- D3 receptor agonists 7-OH-DPAT and PD 128907 following repetitive amphetamine administration. *Neuropsychopharmacology*, 28: 1422–1432.
- Richtand, N.M., Woods, S.C., Berger, S.P. and Strakowski, S.M. (2001b) D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci. Biobehav. Rev.*, 25: 427–443.
- Roth, B.L., Hanizavareh, S.M. and Blum, A.E. (2004) Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology (Berl.)*, 174: 17–24.
- Rueter, L.E., Ballard, M.E., Gallagher, K.B., Basso, A.M., Curzon, P. and Kohlhaas, K.L. (2004) Chronic low dose risperidone and clozapine alleviate positive but not negative symptoms in the rat neonatal ventral hippocampal lesion model of schizophrenia. *Psychopharmacology (Berl.)*, 176: 312–319.
- Sams-Dodd, F., Lipska, B.K. and Weinberger, D.R. (1997) Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood. *Psychopharmacology (Berl.)*, 132: 303–310.
- Schotte, A., Janssen, P.F., Gommeren, W., Luyten, W.H., Van Gompel, P., Lesage, A.S., De Loore, K. and Leysen, J.E. (1996) Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology (Berl.)*, 124: 57–73.
- Sesack, S.R. and Pickel, V.M. (1990) In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. *Brain Res.*, 527: 266–279.
- Shi, L., Fatemi, S.H., Sidwell, R.W. and Patterson, P.H. (2003) Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J. Neurosci.*, 23(1): 297–302.
- Sigala, S., Missale, C. and Spano, P. (1997) Opposite effects of dopamine D2 and D3 receptors on learning and memory in the rat. *Eur. J. Pharmacol.*, 336: 107–112.
- Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.L. and Schwartz, J.C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature*, 347: 146–151.
- Sokoloff, P., Martres, M.P., Giros, B., Bouthenet, M.L. and Schwartz, J.C. (1992) The third dopamine receptor (D3) as a novel target for antipsychotics. *Biochem. Pharmacol.*, 43: 659–666.
- Sorensen, S.M., Kehne, J.H., Fadayel, G.M., Humphreys, T.M., Ketteler, H.J., Sullivan, C.K., Taylor, V.L. and Schmidt, C.J. (1993) Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.*, 266: 684–691.
- Spano, P.F., Govoni, S. and Trabucchi, M. (1978) Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. *Adv. Biochem. Psychopharmacol.*, 19: 155–165.
- Strange, P.G. (1988) The structure and mechanism of neurotransmitter receptors. Implications for the structure and function of the central nervous system. *Biochem. J.*, 249: 309–318.
- Swerdlow, N.R., Halim, N., Hanlon, F.M., Platten, A. and Auerbach, P.P. (2001) Lesion size and amphetamine hyperlocomotion after neonatal ventral hippocampal lesions: more is less. *Brain Res. Bull.*, 55: 71–77.
- Swerdlow, N.R., Lipska, B.K., Weinberger, D.R., Braff, D.L., Jaskiw, G.E. and Geyer, M.A. (1995) Increased sensitivity to the sensorimotor gating-disruptive effects of apomorphine after lesions of medial prefrontal cortex or ventral hippocampus in adult rats. *Psychopharmacology (Berl.)*, 122: 27–34.
- Tsai, G. and Coyle, J.T. (2003) Glutamatergic mechanisms in schizophrenia. *Annu. Rev. Pharmacol. Toxicol.*, 42: 165–179.
- Ugedo, L., Grenhoff, J. and Svensson, T.H. (1989) Ritanerlin, a 5-HT2 receptor antagonist, activates midbrain dopamine neurons by blocking serotonergic inhibition. *Psychopharmacology (Berl.)*, 98: 45–50.
- Varty, G.B., Bakshi, V.P. and Geyer, M.A. (1999) M100907, a serotonin 5-HT2A receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague–Dawley and Wistar rats. *Neuropsychopharmacology*, 20: 311–321.
- Vogel, M., Busse, S., Freyberger, H.J. and Grabe, H.J. (2006) Dopamine D3 receptor and schizophrenia: a widened scope for the immune hypothesis. *Med. Hypotheses*, 67: 354–358.
- Wallace, D.R., Mactutus, C.F. and Booze, R.M. (1996) Repeated intravenous cocaine administration: locomotor activity and dopamine D2/D3 receptors. *Synapse*, 23: 152–163.
- Wan, R.Q. and Corbett, R. (1997) Enhancement of postsynaptic sensitivity to dopaminergic agonists induced by neonatal hippocampal lesions. *Neuropsychopharmacology*, 16: 259–268.
- Wan, R.Q., Giovanni, A., Kafka, S.H. and Corbett, R. (1996) Neonatal hippocampal lesions induced hyperresponsiveness to amphetamine: behavioral and in vivo microdialysis studies. *Behav. Brain Res.*, 78: 211–223.
- Wassef, A.A., Dott, S.G., Harris, A., Brown, A., O'Boyle, M., Meyer, W.J. and Rose, R.M. (1999) Critical review of GABA-ergic drugs in the treatment of schizophrenia. *J. Clin. Psychopharmacol.*, 19: 222–232.
- Waters, N., Svensson, K., Haadsma-Svensson, S.R., Smith, M.W. and Carlsson, A. (1993) The dopamine D3-receptor: a postsynaptic receptor inhibitory on rat locomotor activity. *J. Neural. Transm. Gen. Sect.*, 94: 11–19.
- Woods, S.W., Breier, A., Zipursky, R.B., Perkins, D.O., Addington, J., Miller, T.J., Hawkins, K.A., Marquez, E., Lindborg, S.R., Tohen, M. and McGlashan, T.H. (2003) Randomized trial of olanzapine versus placebo in the symptomatic acute treatment of the schizophrenic prodrome. *Biol. Psychiatry*, 54: 453–464.
- Xia, Z., Gray, J.A., Compton-Toth, B.A. and Roth, B.L. (2003) A direct interaction of PSD-95 with 5-HT2A serotonin receptors regulates receptor trafficking and signal transduction. *J. Biol. Chem.*, 278: 21901–21908.
- Xu, M., Koeltzow, T.E., Santiago, G.T., Moratalla, R., Cooper, D.C., Hu, X.T., White, N.M., Graybiel, A.M., White, F.J. and Tonegawa, S. (1997) Dopamine D3 receptor

- mutant mice exhibit increased behavioral sensitivity to concurrent stimulation of D1 and D2 receptors. *Neuron*, 19: 837–848.
- Zicha, J. and Kunes, J. (1999) Ontogenetic aspects of hypertension development: analysis in the rat. *Physiol. Rev.*, 79: 1227–1282.
- Zuckerman, L., Rehavi, M., Nachman, R. and Weiner, I. (2003) Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. *Neuropsychopharmacology*, 28: 1778–1789.

CHAPTER 8

Role of serotonin and dopamine receptor binding in antipsychotic efficacy

Neil M. Richtand^{1,2,*}, Jeffrey A. Welge^{2,3}, Aaron D. Logue^{1,2}, Paul E. Keck Jr.²,
Stephen M. Strakowski² and Robert K. McNamara²

¹Cincinnati Veterans Affairs Medical Center, 3200 Vine Street, ML0559, Cincinnati, OH 45220, USA

²Department of Psychiatry, University of Cincinnati College of Medicine, 231 Albert Sabin Way, ML0559, Cincinnati, OH 45267-0559, USA

³Center for Biostatistical Services, University of Cincinnati College of Medicine, 231 Albert Sabin Way, ML0559, Cincinnati, OH 45267-0559, USA

Abstract: In an effort to analyse the contribution of individual serotonin and dopamine receptor subtypes to antipsychotic medication response, we analysed the correlation between clinically effective antipsychotic drug dose and binding affinity to cloned serotonin and dopamine receptor subtypes. Clinically effective dosage and binding affinity to the D₂ dopamine receptor subtype were moderately correlated for typical antipsychotic medications ($r=0.57$, $p=0.04$), and were similarly modestly correlated for atypical antipsychotic drugs ($r=0.66$, $p=0.07$). Surprisingly for typical antipsychotic medications, a stronger *inverse* correlation was observed between drug dosage and 5-HT_{2C} affinity ($r=-0.65$, $p=0.03$). The strongest correlation observed for typical antipsychotic medications was between medication dose and 5-HT_{2C}/D₂ binding affinity ratio ($r=-0.81$, $p=0.002$). For atypical antipsychotic medications, highly significant correlations were observed between medication dose and receptor-binding affinity to D₃ dopamine receptor ($r=0.78$, $p=0.02$), and with the ratios of D₂/5-HT_{1A} ($r=0.85$, $p=0.009$), D₃/5-HT_{1A} ($r=0.78$, $p=0.021$), D₂ (5-HT_{2A}/5-HT_{1A}) ($r=0.75$, $p=0.033$) and D₃ (5-HT_{2A}/5-HT_{1A}) ($r=0.75$, $p=0.03$) receptor-binding affinities. The correlation between medication dose and D₂ (5-HT_{2C}/5-HT_{1A}) receptor-binding affinity ratio was of similar magnitude ($r=0.70$, $p=0.055$). No significant correlations were identified between atypical antipsychotic medication dose and 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{2C}/D₂ or 5-HT_{2A}/D₂ receptor-binding affinities. These observations suggest an interaction between D₂ and 5-HT_{2C} receptor-binding effects contributing to the therapeutic response achieved following treatment with typical antipsychotic medications. This suggests that for typical antipsychotic medications, constitutive serotonin 5-HT_{2C} receptor signalling interacts with and facilitates the antipsychotic benefit achieved through dopamine D₂ receptor blockade. Additionally, this analysis demonstrates that, in contrast to typical antipsychotic medications, the therapeutic effectiveness of atypical antipsychotic medications results from opposing interactions among three distinct domains: (1) antipsychotic potency is enhanced by increased D₂ and D₃ dopamine receptor-binding affinity; (2) antipsychotic efficacy is also facilitated by increased binding

*Corresponding author. Tel.: +1 513 558 6657;
Fax: +1 513 558 0042; E-mail: Neil.Richtand@uc.edu

affinity to serotonin 5-HT_{2C} and 5-HT_{2A} receptors; (3) in contrast, antipsychotic potency is reduced by elevations in 5-HT_{1A} receptor-binding affinity.

Keywords: dopamine; serotonin; schizophrenia; psychosis; neuroleptic; antipsychotic

Introduction

More than three decades after Philip Seeman and Ian Creese first identified the direct relationship between dopamine (DA) D₂ receptor-binding affinity and antipsychotic drug potency (Creese et al., 1976; Seeman et al., 1976), this correlation remains an important foundation of hypotheses for both the aetiology and the treatment of psychotic disorders (Seeman and Talerico, 1998; Emilien et al., 1999; Seeman, 2002). Over the past 30 years, however, important advances have been made in the medications, and clinically effective drug dosages, used in the treatment of psychosis. Our understanding of the receptor subtypes comprising the binding affinities used in earlier analyses has also significantly evolved. Each of these advancements could potentially influence the correlations identified in the earlier investigations.

For example, the DA D₂-family receptor-binding activity assayed in earlier studies (Creese et al., 1976; Seeman et al., 1976) is now known to be comprised of at least three receptor subtypes, termed DA D₂ (Bunzow et al., 1988), DA D₃ (Sokoloff et al., 1990) and DA D₄ receptors (Van Tol et al., 1991). Each of these individual receptor subtypes has now been cloned, and binding data for antipsychotic medications to the individual cloned DA receptor subtypes and their splice variants, which were not available in 1976, can now be utilized. Variability in antipsychotic medication binding to individual D₂-family receptor subtypes (i.e. D₂, D₃ and D₄) could help to elucidate the contribution of individual DA receptor subtypes to the treatment of psychosis.

Additionally, cloning and pharmacological characterization of multiple serotonin (5-HT) receptor subtypes, including the 5-HT_{1A} (Fargin et al., 1988), 5-HT_{2A} (Pritchett et al., 1988) and 5-HT_{2C} (Julius et al., 1988) 5-HT receptor, allow the opportunity to study the relationship between antipsychotic binding affinities to 5-HT receptor

subtypes and clinically effective antipsychotic drug dosages, something that was impossible in 1976. The degree to which D₂ family (Kapur and Remington, 2001) compared with serotonergic (5-HT) receptor binding (Meltzer, 1999) may contribute to the antipsychotic properties of atypical antipsychotic medications is a topic of considerable interest.

Third, newer second-generation atypical antipsychotic medications have been developed and received Food and Drug Administration (FDA) approval since the 1970s, and can now be added to the earlier analyses. Only one second-generation antipsychotic drug, clozapine, had been developed at the time of the original analyses in 1976.

In addition, commonly prescribed doses of antipsychotic medications have changed significantly over the past 25 years (Vuckovic et al., 1990; Baldessarini et al., 1993). Although few dose-finding studies are adequately powered to clearly identify minimally effective antipsychotic drug dosages (Zimbroff et al., 1997), currently available data (Bollini et al., 1994; Geddes et al., 2000), as well as revisions in clinical practice, have resulted in a reduction in the average prescribed dosages of most of the older typical D₂ receptor antagonists since 1976.

And finally, several frequently prescribed antipsychotic medications, including loxapine and perphenazine, were not included in the original Seeman analysis, while an antidepressant medication (trazodone) was included (Seeman et al., 1976). For those reasons, we re-examined the correlation between average antipsychotic drug dosages used in the treatment of psychosis and 5-HT and DA receptor subtype binding in an analysis which included all commonly prescribed antipsychotic medications (Richtand et al., 2007). Here, we report the results of an extension of that analysis which also includes data from paliperidone, the most recently FDA-approved antipsychotic medication.

Methods

Drug affinity K_i values determined by the National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP) (Roth et al., 2004) were utilized in our analysis in order to minimize assay condition variability which may result in differences in K_i value observed for a given receptor (Strange, 2001). K_i values chosen for analysis were those listed as NIMH PDSP assay-certified data, determined from assays using the cloned human receptors with drug of interest as test ligand. For K_i values for which PDSP-certified assay data were not listed, the average K_i value from assay data compiled on the PDSP website (Roth et al., 2004) using the cloned human receptor with drug of interest as the test ligand was utilized. K_i values from cloned human receptor for three drug/receptor combinations not listed in the PDSP database were identified from published

literature. K_i values used in our analysis, with data source, are listed in Tables 1 and 2. As noted, all of the binding data analysed in our study have been previously reported by other investigators.

Average daily antipsychotic drug dose was determined from data in randomized, controlled clinical trials where possible (Leucht et al., 1999), supplemented by the recommended dosage ranges from the ePocrates Rx drug reference guide (ePocrates, San Carlos, CA). The midpoint of the dose range was utilized in subsequent calculations. Values for antipsychotic drug dose were established before any data analysis, blind to specific K_i values, and are listed in Table 3.

Data analysis

Antipsychotic doses and binding affinities were log-transformed before analysis. Linear regression was used to estimate the association between these

Table 1. Antipsychotic medication dopamine receptor K_i values

Drug	Clinically effective dose (mg)	K_i values (nM)								
		D ₁	D ₂	D ₂ short	D ₂ long	D ₃	D ₄	D _{4.2}	D _{4.4}	D ₅
Amisulpride	400–800		<i>1.3</i>			<i>2.4</i>	<i><u>≥1000</u></i>			
Aripiprazole	10–15	387	0.95		<i>0.74</i>	4.5	<i>>1000</i>			1676
Benperidol	12–16		<i>0.027</i>				<i>0.066</i>			
Chlorpromazine	200–800	112	2.0		<i>5.4</i>	5.0	<i>10.8</i>	26.2	<i>15.9</i>	133
Chlorprothixene	50–400		<i>3.3</i>				<i>0.64</i>			
Clozapine	300–600	189	431	<i>143.3</i>	<i>196</i>	646	22.5	45.2	30	235
Fluphenazine	2.5–10	24	0.54			3	35			12
Haloperidol	1–10	83	2	<i>1.21</i>	<i>2.34</i>	12	3.88	6.93	15	147
Loxapine	60–100	54	10		<i>22.3</i>	30	<i>10.9</i>	<i>14</i>	<i>5.9</i>	75
Mesoridazine	100–400		<i>4.3</i>			<i>2.6</i>	<i>9.1</i>			
Molindone	15–150		<i>17.8</i>			<i>47.7</i>	<i>3433</i>			
Olanzapine	10–15	58	72	<i>34.6</i>	<i>33.2</i>	63	<i>17.1</i>	<i>44.2</i>	<i>40.5</i>	90
Paliperidone	6	41.035	9.4			2.6	54.3			29
Perphenazine	16–64		<i>0.56</i>			<i>0.43</i>	<i>28.5</i>			
Pimozide	2–10	<i>>10,000</i>	<i>2.51</i>			<i>2.84</i>	<i>1.8</i>			
Quetiapine	150–750	712	567	555	702	483	2276	1233		1738
Remoxipride	200–400		<i>243</i>		<i>125</i>	<i>1109</i>	<i>2527</i>			
Risperidone	1–4	60.6	4.9	<i>4.73</i>	<i>6.0</i>	12.2	<i>7.12</i>	<i>16.7</i>	<i>26.3</i>	16
Sertindole	12–24		<i>4.14</i>	<i>5.8</i>	<i>4.87</i>	<i>5.76</i>	<i>9.29</i>	<i>17.67</i>		
Thioridazine	200–800	89	10	8.6		53	<i>10.65</i>			216
Thiothixene	4–15	51	1.4			185	6.4	548		261
Trifluoperazine	4–10		<i>1.12</i>			0.45	38	178		
Ziprasidone	140–160	30	4.0	4.2	4.6	17	500.8	35.3		152

Note: Normal font, PDSP-certified data; italic font, PDSP K_i database mean; italic underlined, Schoemaker et al. (1997); italic bold, Seeman and Tallerico (1998).

Table 2. Antipsychotic medication serotonin receptor K_i values

Drug	Clinically effective dose (mg)	K_i values (nM)											
		5-HT _{1A}	5-HT _{1B}	5-HT _{1D}	5-HT _{1E}	5-HT _{1F}	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₃	5-HT _{5A}	5-HT ₆	5-HT ₇
Aripiprazole	10–15	5.6	833	63	8000		17.5	0.36	22.4	628	1241	574	10
Chlorpromazine	200–800	3115	1489	452	344		3.32		15.55	977	118	12	21
Chlorprothixene	50–400						0.43						
Clozapine	300–600	105	398	2132	966	130	9.15	7.38	14.9	241	3857	17	18
Fluphenazine	2.5–10	145	334	334	540		21		983	>10,000	145	28	8
Haloperidol	1–10	1202	165	7606	>10,000	>5000	118.6	1204	5580	>10,000	2247	3666	378
Loxapine	60–100	2456	388	3468	1399		4.38		13.3	190	776	33	88
Mesoridazine	100–400								157			380	
Molindone	15–150	3797					4653		10,000			1008	3053
Olanzapine	10–15	2063	509	1582	2408	310	4.90	11.8	14.2	202	1212	6.0	105
Paliperidone	6	637.8	108.7	15.01	>10,000		1.9	61.86	48	>10,000	277.9	2414	2.7
Perphenazine	16–64	421					5.6		132			17	23
Pimozide	2–10	650					48.35		2112			71	0.5
Quetiapine	150–750	431	1109	>10,000	2402	2240	526		1843	>10,000	3120	1864	308
Remoxipride	200–400						6225						
Risperidone	1–4	427	53.6	29.2	>10,000	1240	0.481	41.6	33.4	>10,000	205.8	2241	6.6
Sertindole	12–24	280	60	96	430	360	0.387		0.9			5.4	28
Thioridazine	200–800	108	109	579	194		21.5		53	>10,000	364	57	99
Thiothixene	4–15	410	151	659	>10,000		50		1356	1863	361	208	15
Trifluoperazine	4–10	950					74		378			144	291
Ziprasidone	140–160	76	4	9	1279		0.73		13	>10,000	291	61	6

Note: Normal font, PDSP-certified data; italic font, PDSP K_i database mean.

Table 3. Correlation between clinically effective antipsychotic dose and receptor binding affinity

Drug	D ₂			D ₃			D ₄			5-HT _{1A}			5-HT _{2A}			5-HT _{2C}		
	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value
Typical	13	0.57	0.04	12	0.35	0.27	13	0.18	0.56	10	0.18	0.62	12	−0.03	0.91	11	−0.65	0.03
Atypical	8	0.66	0.07	8	0.78	0.02	8	0.49	0.22	8	0.16	0.70	8	0.57	0.14	8	0.33	0.42

Note: Bold font indicates *p*-value < 0.05.

quantities. A binary variable indicating antipsychotic class (typical or atypical) was entered into the model and allowed to interact with (log) dose, so that a different regression equation was estimated for each antipsychotic class. Data were analysed using separate univariate analyses for each receptor subtype. It was not feasible to analyse data using multivariate methods because the limited number of drugs for which K_i values were available for all receptor subtypes examined did not allow for a meaningful statistical analysis. The linear correlation coefficient (*r*) is reported as a standardized measure of strength of association for the regressions within each class.

Results

The correlation between average clinically effective antipsychotic dose and binding affinity to the cloned human D₂ receptor is illustrated in Table 3 and Fig. 1. Clinically effective dose and binding affinity to D₂ DA receptor were similarly correlated for typical antipsychotic medications (*r* = 0.57, *p* = 0.04), and for second-generation antipsychotic medications (*r* = 0.66, *p* = 0.07).

The relationship between average clinically effective dose and binding affinity to the cloned human D₃ receptor is shown in Table 3 and Fig. 2. There is no clear correlations between these

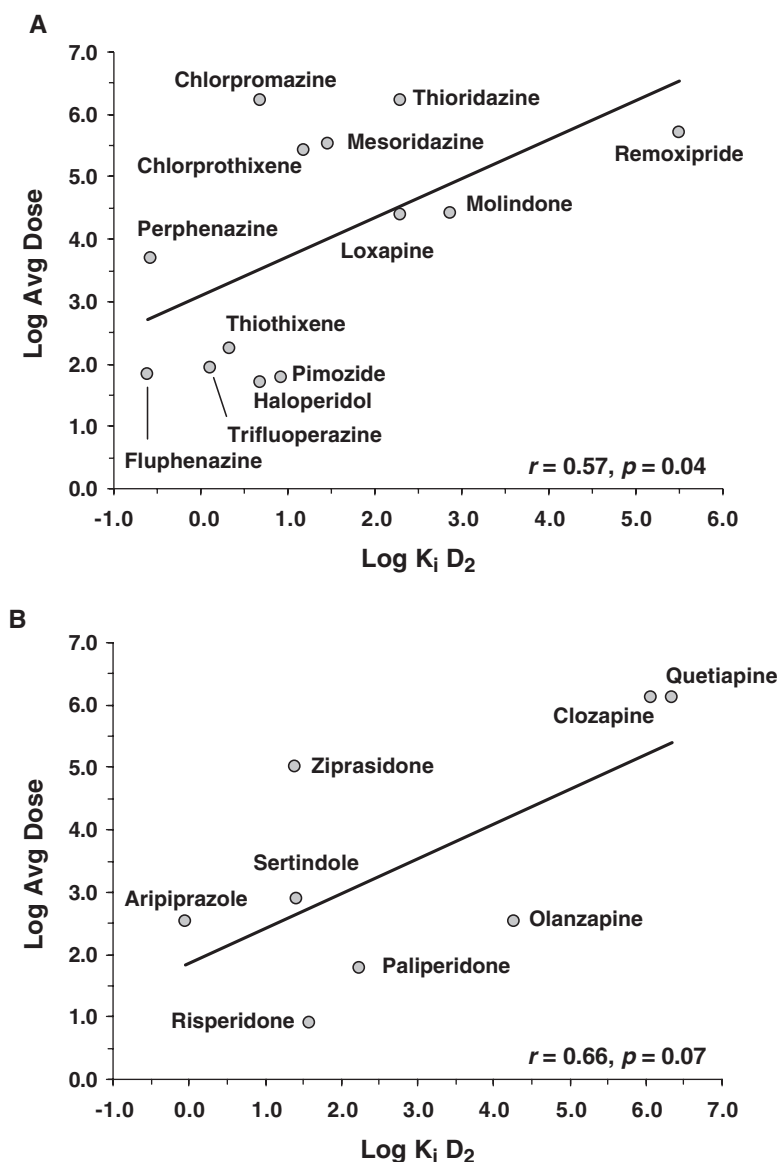


Fig. 1. Clinically effective antipsychotic dose vs. binding affinity to cloned human dopamine D_2 receptor for (A) typical and (B) atypical antipsychotic medications.

variables for typical antipsychotic medications ($r = 0.35$, $p = 0.27$); however, for atypical antipsychotic medications, these measures are significantly correlated ($r = 0.78$, $p = 0.02$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human D_4 receptor is shown in Table 3. For typical antipsychotic drugs, there

was no correlation between these two measures ($r = 0.18$, $p = 0.56$). For atypical antipsychotics, the relationship between the two measures did not reach statistical significance ($r = 0.49$, $p = 0.22$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human 5-HT_{1A} receptor is shown in

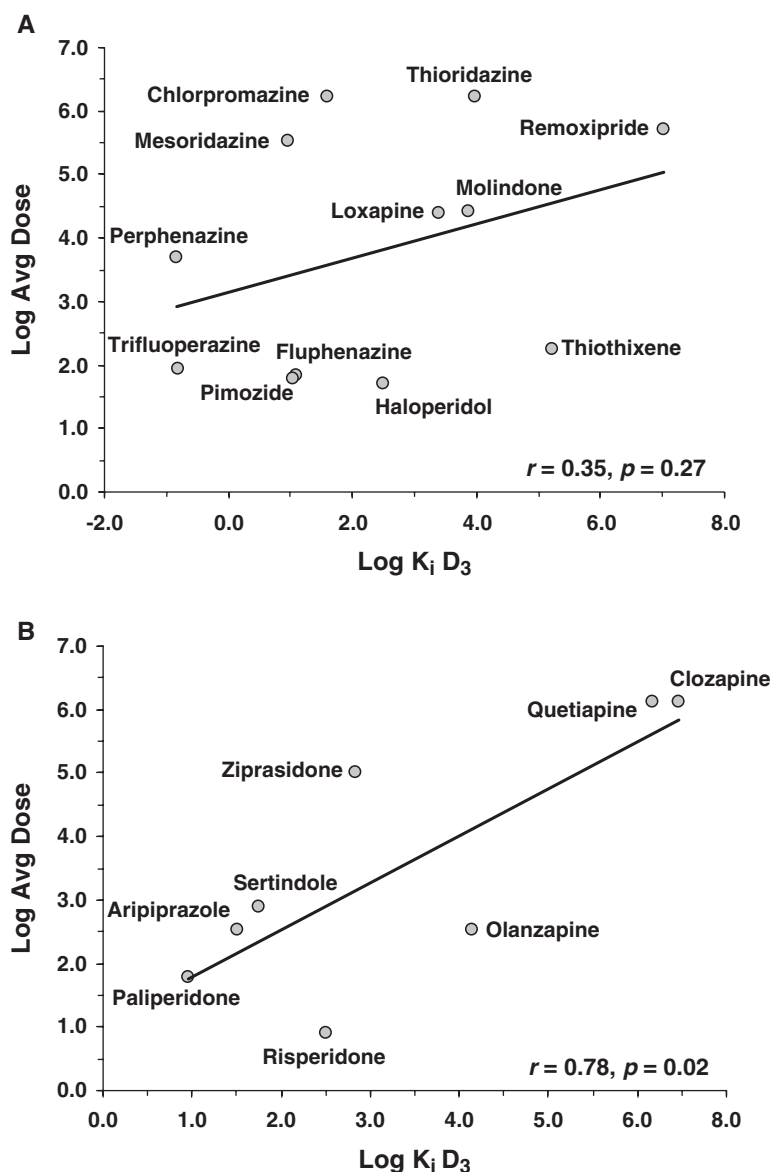


Fig. 2. Clinically effective antipsychotic dose vs. binding affinity to cloned human dopamine D_3 receptor for (A) typical and (B) atypical antipsychotic medications.

Table 3 and Fig. 3. There were no detectable direct relationships between clinically effective antipsychotic dose and receptor-binding affinity for typical ($r = 0.18, p = 0.62$) or atypical antipsychotic medications ($r = -0.16, p = 0.70$).

The relationship between average clinically effective antipsychotic dose and binding affinity to the cloned human $5-HT_{2A}$ receptor is shown in

Table 3 and Fig. 4. There is no direct relationship between clinically effective antipsychotic dose and receptor-binding affinity for typical antipsychotic medications ($r = -0.03, p = 0.91$). In contrast, for atypical antipsychotic medications, binding affinity and clinically effective dose were non-significantly correlated, with a moderate effect size ($r = 0.57, p = 0.14$).

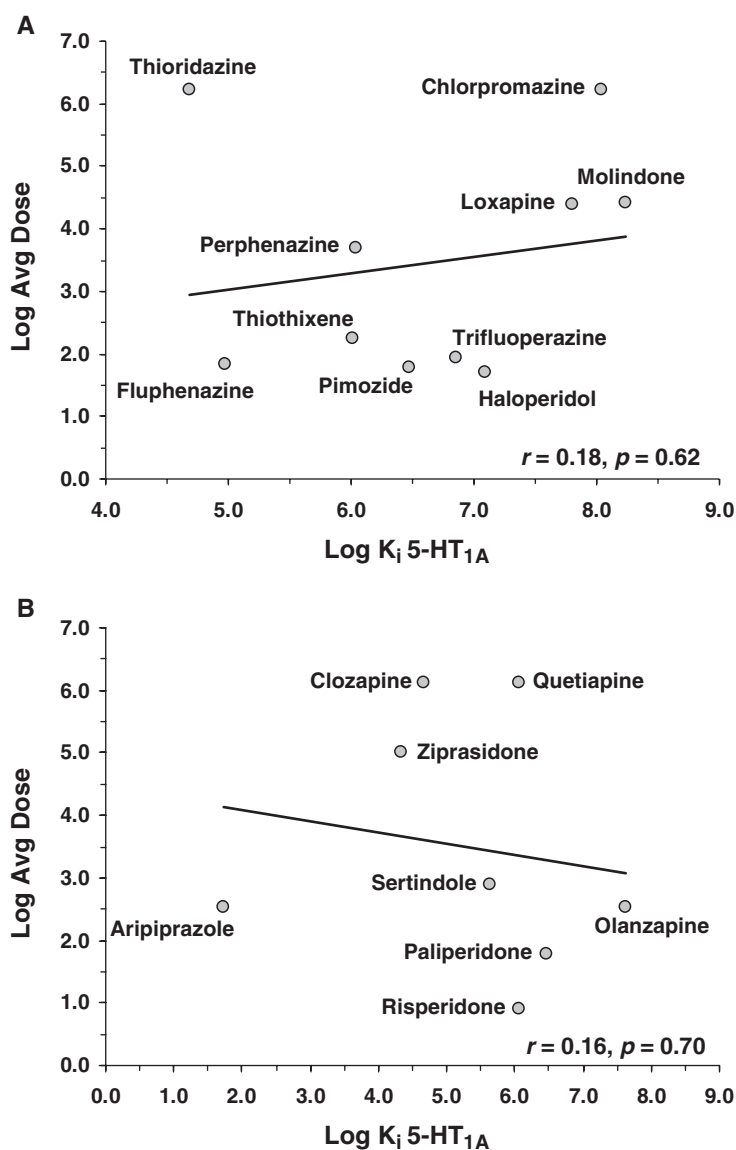


Fig. 3. Clinically effective antipsychotic dose vs. binding affinity to cloned human serotonin 5-HT_{1A} receptor for (A) typical and (B) atypical antipsychotic medications.

The relationship between average clinically effective dose and binding affinity to the cloned human 5-HT_{2C} receptor is shown in Table 3 and Fig. 5. For typical antipsychotic drugs, binding affinity and clinically effective dose were surprisingly *negatively* correlated ($r = -0.65$, $p = 0.03$, Fig. 5A). For atypical antipsychotic medications, the direction of the correlation was *opposite*

(Fig. 5B), and there was no significant direct relationship between clinically effective antipsychotic dose and binding affinity ($r = 0.33$, $p = 0.42$).

In order to evaluate possible interactions between receptor subtypes playing a role in mechanism of antipsychotic efficacy, we analysed correlations between log(average dose) and log(ratio of binding affinities) for combinations of individual receptor

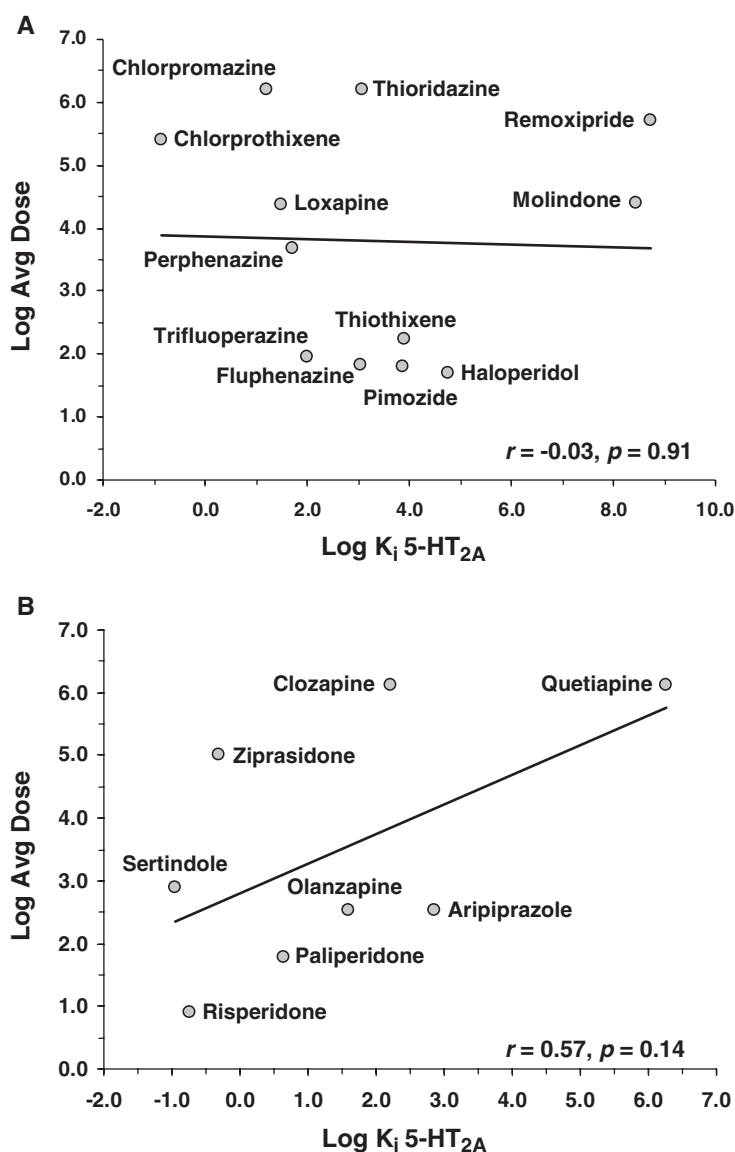


Fig. 4. Clinically effective antipsychotic dose vs. binding affinity to cloned human serotonin 5-HT_{2A} receptor for (A) typical and (B) atypical antipsychotic medications.

subtypes, as shown in Tables 4 and 5. As illustrated in Table 4 and Fig. 6A, there is no correlation between average clinically effective dose and ratio of binding affinities for D₂/5-HT_{1A} receptors for typical antipsychotic medication ($r = 0.33, p = 0.35$). In contrast, for atypical antipsychotic medications, there was a highly significant relationship between dose and D₂/5-HT_{1A} binding affinity ratio ($r = 0.85,$

$p = 0.009$, Fig. 6B). Table 4 also lists the binding affinity ratios for D₃/5-HT_{1A} and D₄/5-HT_{1A} receptors. For typical antipsychotic medications, there are no correlations between average clinically effective dose and either the D₃/5-HT_{1A} or D₄/5-HT_{1A} binding affinity ratios. For atypical antipsychotic medications, there was a significant relationship between dose and D₃/5-HT_{1A} binding affinity ratio

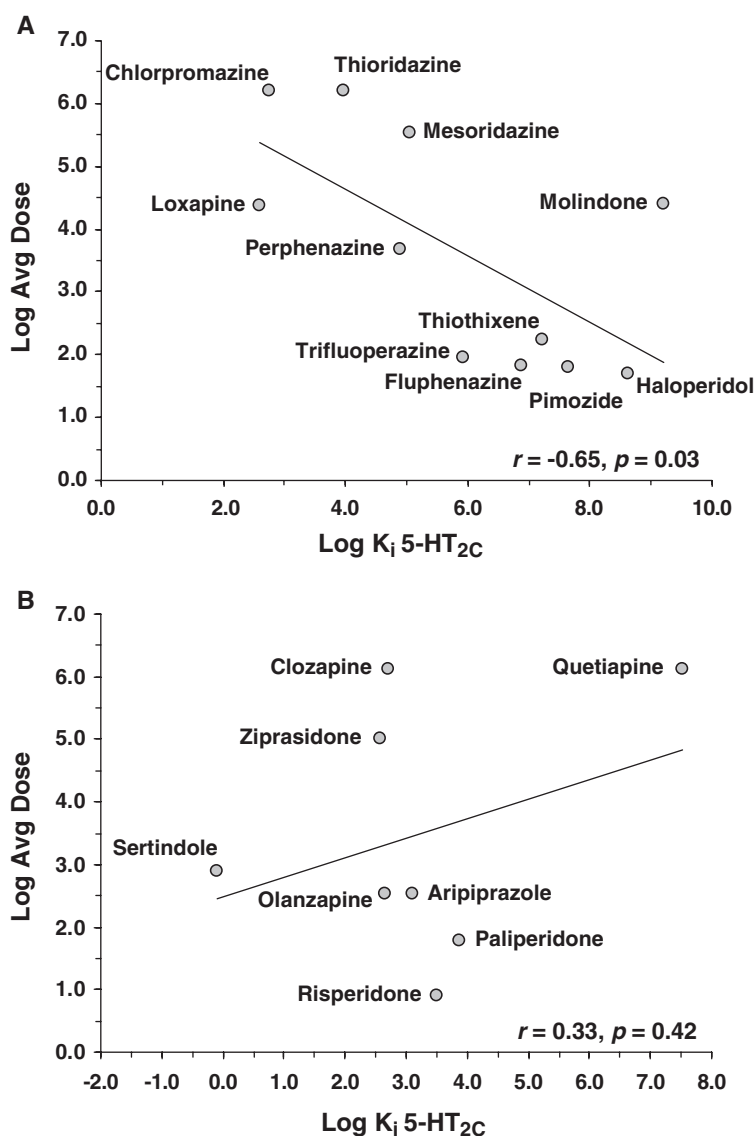


Fig. 5. Clinically effective antipsychotic dose vs. binding affinity to cloned human serotonin 5-HT_{2C} receptor for (A) typical and (B) atypical antipsychotic medications.

($r = 0.78, p = 0.021$), while there was not a significant direct relationship between clinically effective antipsychotic dose and binding affinity ratio for D₄/5-HT_{1A} receptors ($r = 0.40, p = 0.33$).

As illustrated in Table 5 and Fig. 7A, for typical antipsychotic medications, average clinically effective dose and ratio of binding affinities for 5-HT_{2A}/D₂ receptors were not significantly correlated ($r = -0.50,$

$p = 0.10$). For atypical antipsychotics, there was no detectable relationship between dose and 5-HT_{2A}/D₂ binding affinity ratio ($r = -0.11, p = 0.80$, Fig. 7B). Similar analyses of 5-HT_{2A}/D₃ and 5-HT_{2A}/D₄ receptor-binding affinity ratios did not identify significant correlations between these values and clinically effective dosages of typical or atypical antipsychotics (Table 5).

Table 4. Correlation between clinically effective antipsychotic dose and receptor binding affinity ratios

Drug	D ₂ /5-HT _{1A}			D ₃ /5-HT _{1A}			D ₄ /5-HT _{1A}			D ₃ (5-HT _{2A} /5-HT _{1A})			D ₃ (5-HT _{2C} /5-HT _{1A})		
	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value
Typical	10	0.33	0.35	10	0.15	0.67	10	0.12	0.75	10	0.01	0.99	10	-0.37	0.29
Atypical	8	0.85	0.009	8	0.78	0.021	8	0.40	0.33	8	0.75	0.030	8	0.45	0.26

Note: Bold font indicates *p*-value < 0.05.

Table 5. Correlation between clinically effective antipsychotic dose and receptor binding affinity ratios

Drug	5-HT _{2A} /D ₂			5-HT _{2C} /D ₂			5-HT _{2A} /D ₃			5-HT _{2C} /D ₃			5-HT _{2A} /D ₄			5-HT _{2C} /D ₄		
	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value
Typical	12	-0.50	0.10	11	-0.81	0.002	11	-0.36	0.28	11	-0.67	0.025	12	-0.37	0.24	11	-0.71	0.014
Atypical	8	-0.11	0.80	8	-0.39	0.33	8	-0.15	0.72	8	-0.43	0.28	8	0.13	0.75	8	-0.22	0.61
	5-HT _{2A} × D ₂			5-HT _{2C} × D ₂			D ₂ (5-HT _{2A} /5-HT _{1A})			D ₂ (5-HT _{2C} /5-HT _{1A})			D ₂ (5-HT _{1A} /5-HT _{2A})			D ₂ (5-HT _{1A} /5-HT _{2C})		
	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value	<i>n</i>	<i>r</i>	<i>p</i> -Value
Typical	12	0.22	0.50	11	-0.33	0.31	10	0.04	0.91	10	-0.41	0.23	10	0.53	0.11	10	0.77	0.010
Atypical	8	0.68	0.06	8	0.58	0.13	8	0.75	0.033	8	0.70	0.055	8	-0.02	0.96	8	0.17	0.68

Note: Bold font indicates *p*-value < 0.05.

As shown in Table 5 and Fig. 8A, typical antipsychotic medication dose and 5-HT_{2C}/D₂ receptor-binding affinity ratio were strongly and inversely correlated ($r = -0.81$, $p = 0.002$). 5-HT_{2C}/D₃ and 5-HT_{2C}/D₄ receptor-binding affinity ratios were similarly correlated with clinically effective dosages of typical antipsychotic medications (Table 5). In contrast, there was no detectable relationship between dose and 5-HT_{2C}/D₂ receptor-binding affinity ratio for second-generation antipsychotic medications ($r = -0.39$, $p = 0.33$, Fig. 8B). Similarly, 5-HT_{2C}/D₃ and 5-HT_{2C}/D₄ receptor-binding affinity ratios were also not correlated with clinically effective dosages of atypical antipsychotic medications (Table 5).

The relationship between receptor subtype binding and clinical efficacy was further evaluated for atypical antipsychotic medications using a more comprehensive set of binding affinity ratios. While there is not a universal consensus on this point, it has previously been suggested that the antipsychotic effect of atypical antipsychotic medications results from a balance of inhibition at 5-HT_{2A}, 5-HT_{2C} and DA D₂ receptors (Meltzer, 1989, 1995; Leysen et al.,

1993; Huttunen, 1995), coupled with simultaneous agonist effects at 5-HT_{1A} receptors (Protais et al., 1994; Meltzer, 1999; Millan, 2000). In order to identify therapeutic benefit resulting from the interaction between simultaneous effects at these receptor subtypes, we analysed the relationship between clinically effective antipsychotic medication dose and ratios incorporating the binding affinities for each of these receptor systems. As shown in Table 5 and Fig. 9B, atypical antipsychotic medication dose and D₂ (5-HT_{2A}/5-HT_{1A}) binding affinity ratio are highly correlated ($r = 0.75$, $p = 0.033$). A similar relationship was observed between atypical antipsychotic medication dose and D₂ (5-HT_{2C}/5-HT_{1A}) binding affinity ratio ($r = 0.70$, $p = 0.055$, Fig. 10B). Additionally, a highly significant correlation was identified between atypical antipsychotic medication dose and D₃ (5-HT_{2A}/5-HT_{1A}) binding affinity ratio ($r = 0.75$, $p = 0.030$, Table 4). The relationship between D₃ (5-HT_{2C}/5-HT_{1A}) binding affinity ratio and atypical antipsychotic medication dose was not, however, significantly correlated ($r = 0.45$, $p = 0.26$, Table 4).

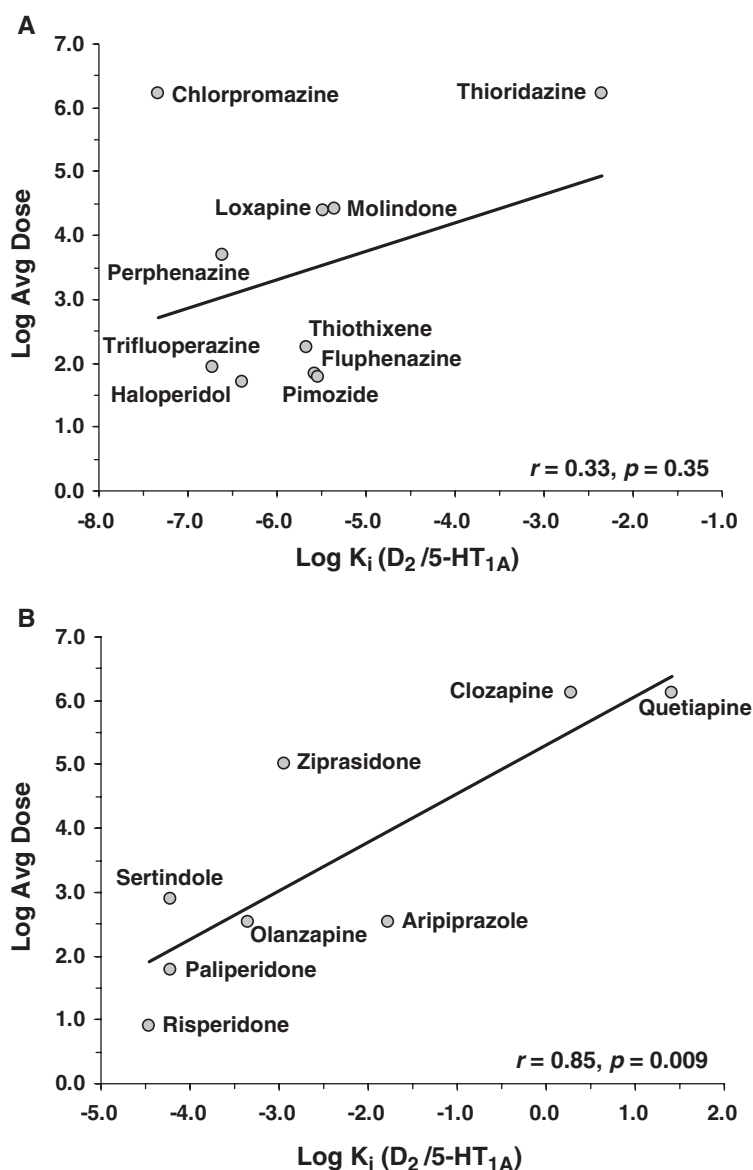


Fig. 6. Clinically effective antipsychotic dose vs. ratio of binding affinity to cloned human dopamine D_2 /serotonin 5-HT_{1A} receptor for (A) typical and (B) atypical antipsychotic medications.

In contrast, neither D_2 ($5\text{-HT}_{2A}/5\text{-HT}_{1A}$) nor D_2 ($5\text{-HT}_{2C}/5\text{-HT}_{1A}$) binding affinity ratios are significantly correlated with clinically effective dose for typical antipsychotic medications (Figs. 9A and 10A and Table 5). Also, D_3 ($5\text{-HT}_{2A}/5\text{-HT}_{1A}$) and D_3 ($5\text{-HT}_{2C}/5\text{-HT}_{1A}$) binding affinity ratios are not related to clinically effective dose for typical antipsychotic medications (Table 4).

Removing the 5-HT_{1A} receptor-binding affinity term from the equation by correlating antipsychotic medication dose with ($5\text{-HT}_{2A} \times D_2$) or ($5\text{-HT}_{2C} \times D_2$) binding affinity ratio diminishes the resulting degree of correlation (Table 5). Similarly, the receptor-binding relationships can be modified so that 5-HT_{1A} and D_2 receptor binding no longer have functionally opposite

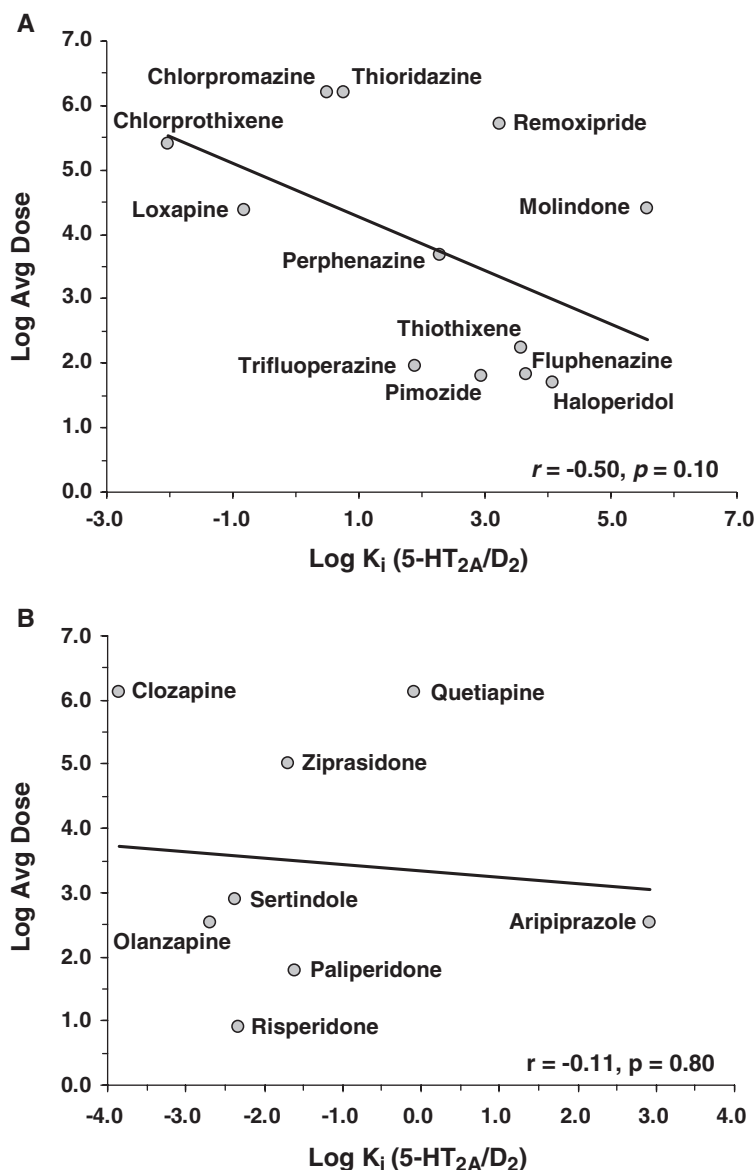


Fig. 7. Clinically effective antipsychotic dose vs. ratio of binding affinity to cloned human serotonin 5-HT_{2A}/dopamine D₂ receptor for (A) typical and (B) atypical antipsychotic medications.

roles, and D₂ binding no longer has a functionally similar action as 5-HT_{2A} and 5-HT_{2C} binding, by inverting the 5-HT receptor affinity terms (Table 5, lower right two columns). This modification completely eliminates the correlation between binding affinity ratio and drug dosage for atypical antipsychotic medications. Typical antipsychotic drug dosage, in contrast,

is significantly correlated with the resulting binding affinity ratio D₂ (5-HT_{1A}/5-HT_{2C}) ($r=0.77$, $p=0.010$, Fig. 5). Comparing this result to the 5-HT_{2C}/D₂ binding affinity versus typical antipsychotic drug dosage correlation described above (Table 5) suggests that the D₂/5-HT_{2C} term contributes the majority of influence to this relationship.

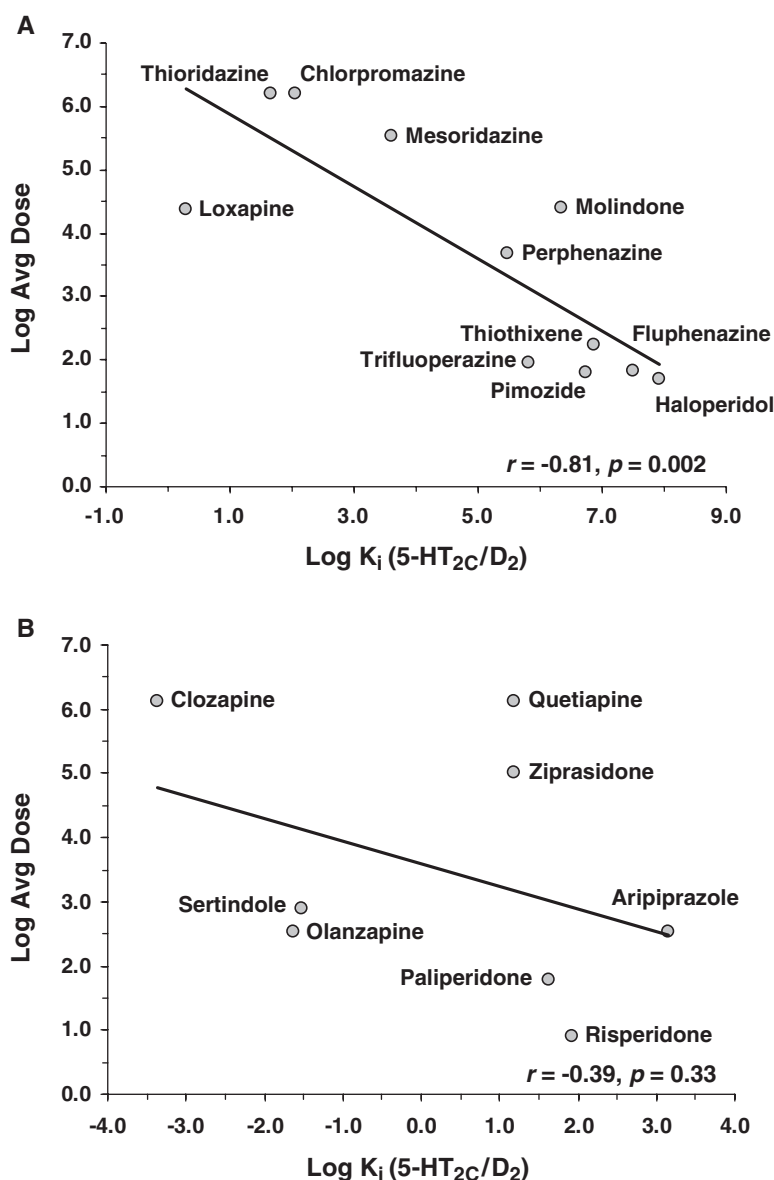


Fig. 8. Clinically effective antipsychotic dose vs. ratio of binding affinity to cloned human serotonin 5-HT_{2C}/dopamine D₂ receptor for (A) typical and (B) atypical antipsychotic medications.

Discussion

Here we present data extending a prior analysis evaluating the relationship between binding affinity to several catecholamine receptor subtypes and drug dosage for antipsychotic efficacy (Richtand et al., 2007). Our analyses are similar in concept to prior

studies demonstrating a linear correlation between antipsychotic drug dose and D₂-family DA receptor-binding affinity (Creese et al., 1976; Seeman et al., 1976). Our goal was to evaluate additional DA and 5-HT receptor subtypes, which had not been identified at the time of the earlier analyses, in order to determine whether affinity to individual receptor

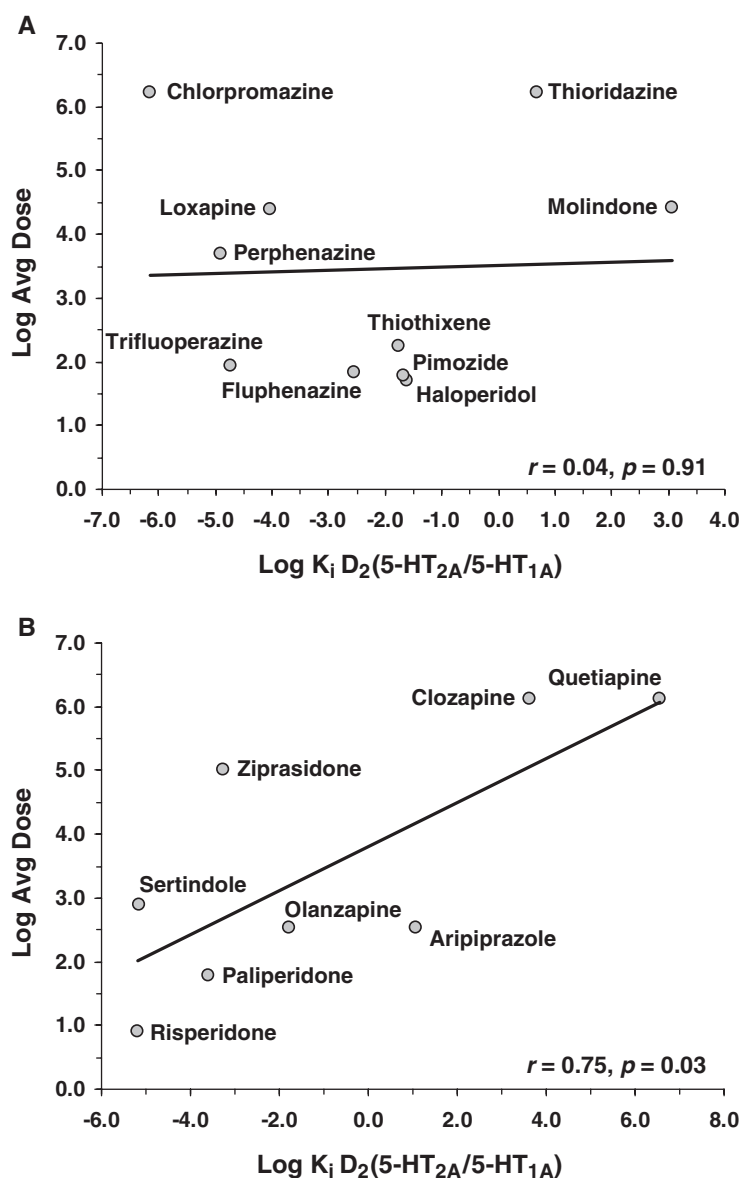


Fig. 9. Clinically effective antipsychotic dose vs. ratio of binding affinity to cloned human D₂ (5-HT_{2A}/5-HT_{1A}) receptor for (A) typical and (B) atypical antipsychotic medications.

subtypes could be correlated with antipsychotic potency of these medications. Although we expected to identify common DA receptor subtypes mediating antipsychotic efficacy for both typical and atypical antipsychotic medications, our analysis instead identified surprising differences in serotonergic mechanisms mediating antipsychotic efficacy for

typical vs. atypical medications. The major findings identified by these analyses are discussed below.

Typical antipsychotic medications

In agreement with earlier studies, we determined that antipsychotic drug dosage for typical

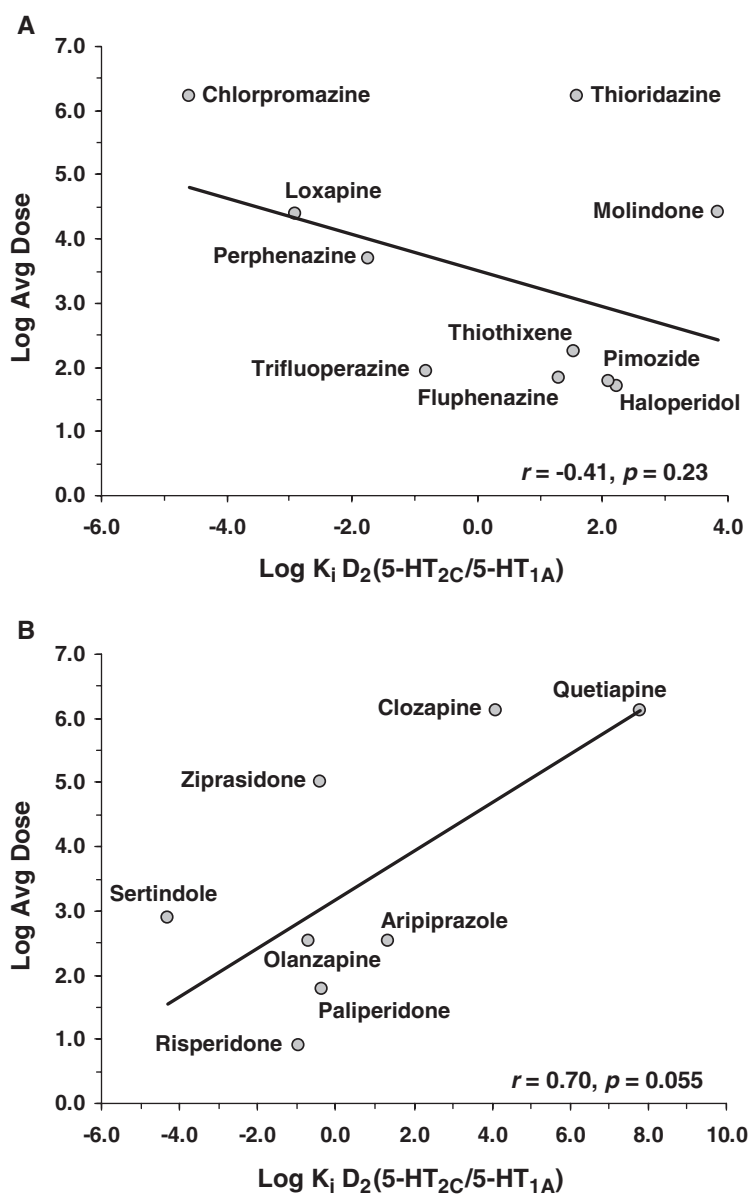


Fig. 10. Clinically effective antipsychotic dose vs. ratio of binding affinity to cloned human D_2 ($5-HT_{2C}/5-HT_{1A}$) receptor for (A) typical and (B) atypical antipsychotic medications.

antipsychotic medications is directly correlated with binding to D_2 DA receptors; however, the strength of this correlation was less robust than anticipated. Our data suggest that this may be related in part to interactions between typical antipsychotic medications and $5-HT_{2C}$ receptors. The observation that $5-HT_{2C}$ receptor affinity is

negatively correlated with antipsychotic drug dosage for *typical* antipsychotic medications was an unexpected outcome of our data analysis. Additionally, $5-HT_{2C}$ and D_2 receptor-binding affinities of typical antipsychotic medications interact such that the ratio of $5-HT_{2C}/D_2$ receptor-binding affinity more accurately predicts

dosage needed for antipsychotic effect than do 5-HT_{2C} or D₂ binding affinities independently. Thus, increasing 5-HT_{2C} receptor antagonist affinity lowers antipsychotic potency at any given level of D₂ blockade, suggesting that signalling through 5-HT_{2C} receptors interacts with and improves antipsychotic effects achieved via D₂ receptor blockade. In contrast, the correlation between 5-HT_{2C} receptor affinity and clinically effective antipsychotic drug dose for *atypical* antipsychotic medications is in the opposite direction, and the degree of correlation is less pronounced (Fig. 5). Although a potential role for 5-HT_{2C} receptor *antagonism* in the therapeutic effect of *atypical* antipsychotic medications has previously been discussed (Meltzer, 1995; Meltzer et al., 2003; Wood et al., 2006), it has also been suggested that 5-HT_{2C} *agonism* could be therapeutic (Meltzer, 1999; Marquis et al., 2007) based on a wide range of preclinical measures demonstrating that 5-HT_{2C} receptor stimulation inhibits the mesolimbic DA system (Millan et al., 1998; De Deurwaerdere and Spampinato, 1999; Di Giovanni et al., 1999; Di Matteo et al., 1999, 2002; Pozzi et al., 2002; Alex et al., 2005). These findings are consistent with our observation, and suggest a potential mechanism for 5-HT_{2C} receptor blockade to worsen psychotic symptoms. Human data supporting the concept that 5-HT_{2C} blockade lowers the antipsychotic potency of first-generation antipsychotic medications have not been previously elucidated to our knowledge, however.

The neuroanatomical mechanism(s) underlying this finding may be related to the tonic inhibitory control exerted by 5-HT operating through 5-HT_{2C} receptors over limbic dopaminergic pathways (De Deurwaerdere and Spampinato, 1999). Serotonergic cell bodies originating in the raphe nucleus project diffusely to targets throughout the brain, and strong 5-HT_{2C} receptor expression has been observed in nucleus accumbens and ventral striatum, with modest expression in prefrontal cortex (Eberle-Wang et al., 1997; Lopez-Gimenez et al., 2001). In prefrontal cortex, 5-HT_{2C} receptors are co-expressed with DA D₄ receptors (Vysokanov et al., 1998). Within the substantia nigra/ventral tegmentum, 5-HT_{2C} receptors are expressed on inhibitory GABA-ergic interneurons

(Eberle-Wang et al., 1997). 5-HT_{2C} receptor stimulation inhibits reward system-related behaviours including cocaine-induced hyperlocomotion (Grottick et al., 2000; Filip and Cunningham, 2003). 5-HT_{2C} receptor blockade increases both dopaminergic cell firing and DA release in nucleus accumbens and frontal cortex (Millan et al., 1998; Di Matteo et al., 1999; Alex et al., 2005). 5-HT_{2C} receptor blockade could oppose the actions of D₂ DA receptor blockade either through direct effects on second messenger systems in neurons co-expressing DA D₂ and 5-HT_{2C} receptors or indirectly through a systems effect on components of limbic neurotransmission. In addition to antagonist effects through inhibition of basal 5-HT tone, constitutive activity of 5-HT_{2C} receptor isoforms has also been previously described (Westphal et al., 1995; Niswender et al., 1999), and this constitutive activity participates in the tonic inhibition of mesolimbic DA function (De Deurwaerdere et al., 2004). Antipsychotic medications could therefore also function as inverse agonists through second messenger pathways of 5-HT_{2C} isoforms (Rauser et al., 2001; Navailles et al., 2006). While previous studies of the 5-HT_{2C} inverse agonist properties of antipsychotic medications have identified the potential role for inverse agonism in the mechanism of action of antipsychotic efficacy (Navailles et al., 2006), our data suggest an alternative possibility that 5-HT_{2C} inverse agonism may also directly oppose acute antipsychotic efficacy. Our analysis supports the concept that 5-HT_{2C} agonists, in contrast, may have therapeutic potential as adjunctive medications to improve antipsychotic efficacy for patients receiving typical antipsychotic medication, and suggests that these medications could have applications for treatment refractory psychosis. The potential therapeutic benefit of 5-HT_{2C} stimulation in inhibiting psychotic symptoms through inhibition of meso-accumbens DA function must be balanced with the potential for worsening of cognitive and negative symptoms through decreased mesocortical DA function.

5-HT_{2C}/D₃ and 5-HT_{2C}/D₄ binding affinity ratios were also correlated with clinically effective antipsychotic medication dose for typical antipsychotic medications. Our data therefore suggest

the likelihood of an interaction between binding at 5-HT_{2C} and DA D₂, D₃ and D₄ receptors in the mechanism of action of typical antipsychotic medications.

Our analysis identifies a modest correlation between antipsychotic drug dosage and the ratio of 5-HT_{2A}/D₂ receptor affinity for typical antipsychotic medications.

Atypical antipsychotic medications

Therapeutic efficacy for atypical antipsychotic medications has been suggested to result from a balance of inhibition at DA D₂, 5-HT_{2A} and 5-HT_{2C} receptors (Meltzer, 1989, 1995; Leysen et al., 1993; Huttunen 1995), while 5-HT_{1A} receptor stimulation appears to contribute to antipsychotic efficacy in rat models (Protais et al., 1994; Millan, 2000; Meltzer et al., 2003). Consistent with these observations, clinically effective dosages of atypical antipsychotic medication are highly correlated with the ratios of D₂ (5-HT_{2A}/5-HT_{1A}) and D₂ (5-HT_{2C}/5-HT_{1A}) receptor-binding affinities. Our data suggest that interactions with D₃ subtype DA receptors may also play an important role in the therapeutic effects of atypical antipsychotic medications. Thus, our analysis suggests that the therapeutic effects of atypical antipsychotic medications are determined by interactions among three different domains: (1) increasing D₂ and D₃ DA receptor-binding affinity enhances antipsychotic potency; (2) increasing 5-HT_{2C} and 5-HT_{2A} receptor-binding affinities also facilitates antipsychotic efficacy; (3) increasing 5-HT_{1A} receptor-binding affinity, in contrast, reduces antipsychotic efficacy.

We are not aware of other studies demonstrating that 5-HT_{2C} receptor blockade has opposite effects in typical and atypical antipsychotic medications. It has previously been suggested, however, that both 5-HT_{2C} antagonism (Meltzer, 1995; Meltzer et al., 2003; Wood et al., 2006) and 5-HT_{2C} receptor stimulation (Meltzer, 1999; Marquis et al., 2007) could facilitate antipsychotic activity. Our data support the view that this seemingly paradoxical finding may result from the relatively higher 5-HT_{2A} receptor blockade in atypical versus typical medications. Thus, simultaneous 5-HT_{2A} and 5-HT_{2C} receptor blockade

may be more effective in mediating antipsychotic effects than blockade of either receptor separately (Meltzer et al., 2003).

Although prior studies have suggested a role for the D₃ receptor as a molecular target for antipsychotic medications based on receptor distribution within limbic brain regions believed to play an important role in psychotic symptoms (Sokoloff et al., 1990, 1992), our observation that D₃ receptor binding more robustly correlates with clinically effective drug dosage than D₂ receptor binding for atypical antipsychotic drugs (Table 3) was unexpected. Also, similar to our findings with typical antipsychotic medications, the strength of the correlations between clinically effective drug dosage and D₂/D₃ receptor binding was less robust than anticipated. Previous studies have determined that atypical antipsychotic medications can be distinguished from typical antipsychotic drugs based on the ratio of 5-HT₂/D₂ binding affinities (Meltzer et al., 1989a, b). Thus, we had anticipated a more direct relationship between D₂ binding and antipsychotic efficacy for second-generation medications, and an interaction between 5-HT_{2A} and D₂ effects. Instead, we observed a modest direct correlation between atypical antipsychotic drug dosage and 5-HT_{2A} receptor binding. Also unexpectedly, we observed a very modest correlation between DA D₄ receptor-binding affinity and atypical antipsychotic drug dosages. It has previously been suggested that a subset of atypical antipsychotic medications derives its efficacy in part from selective effects at D₄ DA receptors (Seeman et al., 1997). Our data do not provide evidence supporting the concept that a simple ratio of binding to 5-HT_{2A} or D₄ receptors accounts for a significant proportion of atypical antipsychotic medication efficacy. Addition of more members to the small but growing number of atypical antipsychotic drug class available for analysis may help to further clarify these issues.

Limitations

Our analyses are limited to antipsychotic medication effects on positive psychotic symptoms, and do not address efficacy for negative symptoms or

cognition which may be more important in terms of long-term functional outcome. Importantly, the strength of correlations between receptor binding and antipsychotic efficacy identified in our analyses is restricted by a wide range of limiting factors. Medication differences in absorption, metabolism, protein binding and the presence of pharmacologically active metabolites all serve to weaken the observed correlations. Additionally, the antipsychotic medication dose prescribed to patients may be determined in part by side effects, and might therefore not accurately reflect the 'ideal' efficacy dose. The paucity of adequately powered clinical trials to determine optimal dose for antipsychotic medications further limits the accuracy of medication dosages employed in our analyses. Also, the binding data used in our analyses, measuring ligand binding to cloned human receptors expressed in cell culture systems, may be distinct from binding to limbic neurotransmitter receptor populations *in vivo*. Differences in receptor phosphorylation, glycosylation and/or dimerization to hetero-oligomers (Nimchinsky et al., 1997; Zawarynski et al., 1998; Lee et al., 2000; Scarselli et al., 2001) between *in vivo* and cell culture systems lacking post-translational machinery could potentially alter receptor-binding affinity. Additionally, atypical antipsychotic medications as a group tend to have more rapid dissociation rates from DA receptors than typical antipsychotics (Kapur and Seeman, 2001), an effect which might further complicate the relationship between receptor affinity and clinically effective drug dose. And finally, this approach is inherently limited by the complexities of brain circuitry in which DA and 5-HT receptors may function as a 'brake' in one brain region, and simultaneously as an 'accelerator' in a different brain region. For example, blockade of D₂ DA autoreceptors in cell body regions of the ventral tegmentum increases both synthesis and release of DA, which could worsen psychotic symptoms, while blockade of postsynaptic D₂ receptors in limbic terminal regions would likely have an opposite behavioural effect. Thus, the dysfunction of schizophrenia, resulting from a complex interaction of multiple receptor and neurotransmitter systems (Carlsson et al., 1999), does not lend

itself ideally to an analysis of isolated receptor systems.

Summary

In summary, we present data demonstrating correlations between clinical efficacies of antipsychotic medications and binding affinities to D₂, D₃, D₄, 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptor subtypes. Given the numerous limitations inherent in this approach (listed above), the strength of correlations described in these analyses suggests that the DA and 5-HT receptor subtypes analysed provide the preponderance of antipsychotic effect of these medications. The specific mechanism(s) underlying this clinical effect, however, remains obscure. The 'disconnect' between the pharmacokinetics of receptor blockade and the extended time lag until clinical benefits suggest antipsychotic efficacy, while initiated through binding to neurotransmitter receptor target(s), is likely the result of a downstream cascade of changes in gene transcription and translation. Studies identifying the specific targets of altered gene transcription resulting from these drug-neurotransmitter receptor interactions would therefore have high likelihood of improving specificity and efficacy of antipsychotic medications.

Abbreviations

5-HT	serotonin
FDA	Food and Drug Administration
NIMH	National Institute of Mental Health
PDSP	Psychoactive Drug Screening Program

Acknowledgements

This work was supported by the Department of Veterans Affairs Medical Research Service, and by NARSAD (NMR). The authors thank Nathan Richtand for editorial assistance in preparing the manuscript.

References

- Alex, K.D., Yavanian, G.J., McFarlane, H.G., Pluto, C.P. and Pehek, E.A. (2005) Modulation of dopamine release by striatal 5-HT_{2C} receptors. *Synapse*, 55: 242–251.
- Baldessarini, R.J., Cohen, B.M. and Teicher, M.H. (1993) Clinical dosing of neuroleptics. *Psychopharmacol. Ser.*, 10: 138–148.
- Bollini, P., Pampallona, S., Orza, M.J., Adams, M.E. and Chalmers, T.C. (1994) Antipsychotic drugs: is more worse? A meta-analysis of the published randomized control trials. *Psychol. Med.*, 24: 307–316.
- Bunzow, J.R., Van Tol, H.H., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A. and Civelli, O. (1988) Cloning and expression of a rat D₂ dopamine receptor cDNA. *Nature*, 336: 783–787.
- Carlsson, A., Waters, N. and Carlsson, M.L. (1999) Neurotransmitter interactions in schizophrenia — therapeutic implications. *Eur. Arch. Psychiatry Clin. Neurosci.*, 249(Suppl. 4): 37–43.
- Creese, I., Burt, D.R. and Snyder, S.H. (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192: 481–483.
- De Deurwaerdere, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24: 3235–3241.
- De Deurwaerdere, P. and Spampinato, U. (1999) Role of serotonin_{2A} and serotonin_{2B/2C} receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73: 1033–1042.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin_{2C/2B} receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91: 587–597.
- Di Matteo, V., Cacchio, M., Di Giulio, C. and Esposito, E. (2002) Role of serotonin_{2C} receptors in the control of brain dopaminergic function. *Pharmacol. Biochem. Behav.*, 71: 727–734.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38: 1195–1205.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384: 233–247.
- Emilien, G., Maloteaux, J.M., Geurts, M., Hoogenberg, K. and Cragg, S. (1999) Dopamine receptors — physiological understanding to therapeutic intervention potential. *Pharmacol. Ther.*, 84: 133–156.
- Fargin, A., Raymond, J.R., Lohse, M.J., Kobilka, B.K., Caron, M.G. and Lefkowitz, R.J. (1988) The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. *Nature*, 335: 358–360.
- Filip, M. and Cunningham, K.A. (2003) Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin_{2C} (5-HT_{2C}) receptors in rat prefrontal cortex. *J. Pharmacol. Exp. Ther.*, 306: 734–743.
- Geddes, J., Freemantle, N., Harrison, P. and Bebbington, P. (2000) Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *BMJ*, 321: 1371–1376.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295: 1183–1191.
- Huttunen, M. (1995) The evolution of the serotonin–dopamine antagonist concept. *J. Clin. Psychopharmacol.*, 15: 4S–10S.
- Julius, D., MacDermott, A.B., Axel, R. and Jessell, T.M. (1988) Molecular characterization of a functional cDNA encoding the serotonin_{1c} receptor. *Science*, 241: 558–564.
- Kapur, S. and Remington, G. (2001) Dopamine D₂ receptors and their role in atypical antipsychotic action: still necessary and may even be sufficient. *Biol. Psychiatry*, 50: 873–883.
- Kapur, S. and Seeman, P. (2001) Does fast dissociation from the dopamine d₂ receptor explain the action of atypical antipsychotics? A new hypothesis. *Am. J. Psychiatry*, 158: 360–369.
- Lee, S.P., O'Dowd, B.F., Ng, G.Y., Varghese, G., Akil, H., Mansour, A., Nguyen, T. and George, S.R. (2000) Inhibition of cell surface expression by mutant receptors demonstrates that D₂ dopamine receptors exist as oligomers in the cell. *Mol. Pharmacol.*, 58: 120–128.
- Leucht, S., Pitschel-Walz, G., Abraham, D. and Kissling, W. (1999) Efficacy and extrapyramidal side-effects of the new antipsychotics olanzapine, quetiapine, risperidone, and sertindole compared to conventional antipsychotics and placebo. A meta-analysis of randomized controlled trials. *Schizophr. Res.*, 35: 51–68.
- Leysen, J.E., Janssen, P.M., Schotte, A., Luyten, W.H. and Megens, A.A. (1993) Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT₂ receptors. *Psychopharmacology (Berl.)*, 112: S40–S54.
- Lopez-Gimenez, J.F., Mengod, G., Palacios, J.M. and Vilario, M.T. (2001) Regional distribution and cellular localization of 5-HT_{2C} receptor mRNA in monkey brain: comparison with [3H]mesulergine binding sites and choline acetyltransferase mRNA. *Synapse*, 42: 12–26.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Jr., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1h]indole]: a novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.

- Meltzer, H.Y. (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology* (Berl.), 99(Suppl.): S18-S27.
- Meltzer, H.Y. (1995) The role of serotonin in schizophrenia and the place of serotonin-dopamine antagonist antipsychotics. *J. Clin. Psychopharmacol.*, 15: 2S-3S.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21: 106S-115S.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159-1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989a) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251: 238-246.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989b) The ratios of serotonin₂ and dopamine₂ affinities differentiate atypical and typical antipsychotic drugs. *Psychopharmacol. Bull.*, 25: 390-392.
- Millan, M.J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J. Pharmacol. Exp. Ther.*, 295: 853-861.
- Millan, M.J., Dekeyne, A. and Gobert, A. (1998) Serotonin (5-HT)_{2C} receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. *Neuropharmacology*, 37: 953-955.
- Navailles, S., De Deurwaerdere, P. and Spampinato, U. (2006) Clozapine and haloperidol differentially alter the constitutive activity of central serotonin_{2C} receptors in vivo. *Biol. Psychiatry*, 59: 568-575.
- Nimchinsky, E.A., Hof, P.R., Janssen, W.G.M., Morrison, J.H. and Schmauss, C. (1997) Expression of dopamine D3 receptor dimers and tetramers in brain and in transfected cells. *J. Biol. Chem.*, 272: 29229-29237.
- Niswender, C.M., Copeland, S.C., Herrick-Davis, K., Emeson, R.B. and Sanders-Bush, E. (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, 274: 9472-9478.
- Pozzi, L., Acconcia, S., Ceglia, I., Invernizzi, R.W. and Samanin, R. (2002) Stimulation of 5-hydroxytryptamine (5-HT_{2C}) receptors in the ventro tegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J. Neurochem.*, 82: 93-100.
- Pritchett, D.B., Bach, A.W., Wozny, M., Taleb, O., Dal Toso, R., Shih, J.C. and Seeburg, P.H. (1988) Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *EMBO J.*, 7: 4135-4140.
- Protais, P., Chagraoui, A., Arbaoui, J. and Mocaer, E. (1994) Dopamine receptor antagonist properties of S 14506, 8-OH-DPAT, raclopride and clozapine in rodents. *Eur. J. Pharmacol.*, 271: 167-177.
- Rauser, L., Savage, J.E., Meltzer, H.Y. and Roth, B.L. (2001) Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. *J. Pharmacol. Exp. Ther.*, 299: 83-89.
- Richtand, N.M., Welge, J.A., Logue, A.D., Keck, P.E., Jr., Strakowski, S.M. and McNamara, R.K. (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. *Neuropsychopharmacology*, 32: 1715-1726.
- Roth, B.L., Lopez, E., Beischel, S., Westkaemper, R.B. and Evans, J.M. (2004) Screening the receptorome to discover the molecular targets for plant-derived psychoactive compounds: a novel approach for CNS drug discovery. *Pharmacol. Ther.*, 102: 99-110.
- Scarselli, M., Novi, F., Schallmach, E., Lin, R., Baragli, A., Colzi, A., Griffon, N., Corsini, G.U., Sokoloff, P., Levenson, R., Vogel, Z. and Maggio, R. (2001) D2/D3 dopamine receptor heterodimers exhibit unique functional properties. *J. Biol. Chem.*, 276: 30308-30314.
- Schoemaker, H., Claustre, Y., Fage, D., Rouquier, L., Chergui, K., Curet, O., Oblin, A., Gonon, F., Carter, C., Benavides, J. and Scatton, B. (1997) Neurochemical characteristics of amisulpride, an atypical dopamine D2/D3 receptor antagonist with both presynaptic and limbic selectivity. *J. Pharmacol. Exp. Ther.*, 280: 83-97.
- Seeman, P. (2002) Atypical antipsychotics: mechanism of action. *Can. J. Psychiatry*, 47: 27-38.
- Seeman, P., Corbett, R. and Van Tol, H.H. (1997) Atypical neuroleptics have low affinity for dopamine D2 receptors or are selective for D4 receptors. *Neuropsychopharmacology*, 16: 93-110.
- Seeman, P., Lee, T., Chau-Wong, M. and Wong, K. (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature*, 261: 717-719.
- Seeman, P. and Tallerico, T. (1998) Antipsychotic drugs which elicit little or no parkinsonism bind more loosely than dopamine to brain D2 receptors, yet occupy high levels of these receptors. *Mol. Psychiatry*, 3: 123-134.
- Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.L. and Schwartz, J.C. (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature*, 347: 146-151.
- Sokoloff, P., Martres, M.P., Giros, B., Bouthenet, M.L. and Schwartz, J.C. (1992) The third dopamine receptor (D3) as a novel target for antipsychotics. *Biochem. Pharmacol.*, 43: 659-666.
- Strange, P.G. (2001) Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol. Rev.*, 53: 119-133.
- Van Tol, H.H., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B. and Civelli, O. (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature*, 350: 610-614.
- Vuckovic, A., Cohen, B.M., Keck, P.E.J. and Shedlack, K.J. (1990) Neuroleptic dosage regimens in psychotic inpatients: a retrospective comparison. *J. Clin. Psychiatry*, 51: 107-109.
- Vysokanov, A., Flores-Hernandez, J. and Surmeier, D.J. (1998) mRNAs for clozapine-sensitive receptors co-localize in rat prefrontal cortex neurons. *Neurosci. Lett.*, 258: 179-182.
- Westphal, R.S., Backstrom, J.R. and Sanders-Bush, E. (1995) Increased basal phosphorylation of the constitutively active

- serotonin 2C receptor accompanies agonist-mediated desensitization. *Mol. Pharmacol.*, 48: 200–205.
- Wood, M.D., Scott, C., Clarke, K., Cato, K.J., Patel, N., Heath, J., Worby, A., Gordon, L., Campbell, L., Riley, G., Davies, C.H., Gribble, A. and Jones, D.N. (2006) Pharmacological profile of antipsychotics at monoamine receptors: atypicality beyond 5-HT_{2A} receptor blockade. *CNS Neurol. Disord. Drug Targets*, 5: 445–452.
- Zawarynski, P., Tallerico, T., Seeman, P., Lee, S.P., O'Dowd, B.F. and George, S.R. (1998) Dopamine D₂ receptor dimers in human and rat brain. *FEBS Lett.*, 441: 383–386.
- Zimbroff, D.L., Kane, J.M., Tamminga, C.A., Daniel, D.G., Mack, R.J., Wozniak, P.J., Seabee, T.B., Wallin, B.A. and Kashkin, K.B. (1997) Controlled, dose-response study of sertindole and haloperidol in the treatment of schizophrenia. Sertindole Study Group. *Am. J. Psychiatry*, 154: 782–791.

CHAPTER 9

In vivo actions of atypical antipsychotic drug on serotonergic and dopaminergic systems

Herbert Y. Meltzer^{*,1} and Mei Huang

Department of Psychiatry Vanderbilt, University School of Medicine, Psychiatric Hospital at Vanderbilt, Nashville, TN 37212, USA

Abstract: Atypical antipsychotic drugs related to clozapine, improve psychosis, cognition and negative symptoms, while producing minimal extrapyramidal side effects, in patients with schizophrenia. This appears to be mediated mainly through the combined effect of relatively more potent blockade of 5-HT_{2A} receptors, located on cortical and hippocampal glutamatergic and GABAergic neurons, as well as cell bodies of the mesolimbic and mesocortical dopamine (DA) neurons, and weaker blockade of D₂ receptors in the ventral and dorsal striatum and pyramidal neurons in cortical areas, as well as the cell bodies of DA neurons. This combination of effects is important to their ability to enhance cortical and hippocampal DA efflux, which, while producing less increase of DA efflux in the striatum. Selective inverse agonists of 5-HT_{2A} receptors alone, or in combination with subthreshold doses of atypical antipsychotic drugs have shown effects similar to those of atypicals in both animal models and clinical trials in patients with schizophrenia. Atypical antipsychotic drugs and 5-HT_{2A} receptor antagonists/inverse agonists have been found to prevent or reverse acute and chronic effects of the N-methyl-D-aspartate non-competitive antagonist, phencyclidine (PCP), including cognitive impairment, in part through enhancing the turnover of DA in cortex. PET, postmortem and genetic studies, as well as clinical studies with 5-HT_{2A} hallucinogens, strongly support the importance of 5-HT_{2A} receptor blockade in the action of atypical antipsychotic drugs. Their 5-HT_{1A} receptor partial agonism, produced directly or indirectly, also contributes to enhancement of efflux of DA in cortical regions. Other serotonergic actions, e.g. 5-HT_{2C}, 5-HT₆ and possibly 5-HT₇ antagonism, may also contribute to their efficacy or, in the case of 5-HT_{2C} antagonism, side effects such as weight gain.

Keywords: serotonin; dopamine; antipsychotic; schizophrenia; microdialysis; clozapine; haloperidol

Introduction

Antipsychotic drugs are best described as typical and atypical antipsychotic drugs (APDs), the distinction being those which do and do not cause catalepsy in rodents at doses which block surrogate markers for antipsychotic activity, e.g. blockade of amphetamine-induced locomotor activity and conditioned avoidance response. The human

^{*}Corresponding author. Tel.: +615-327-7049;
Fax: +615-327-7093; E-mail: herbert.meltzer@vanderbilt.edu

¹Herbert Y. Meltzer is a consultant to Abbott Labs, ACADIA, Eli Lilly, Janssen, Lundbeck, Merck, Pfizer, Solvay, Wyeth as well as a shareholder in ACADIA (pimavanserin).

counterpart has less extrapyramidal symptoms (EPS) at clinically effective doses. The prototypes of typical and atypical APDs are haloperidol and clozapine, respectively. There are various classes of drugs which meet this definition of atypical antipsychotic agents but for the purpose of this article, the term will apply only to those drugs which are, like clozapine, more potent serotonin (5-HT)_{2A} than dopamine (DA) D₂ antagonists. There are many such agents in clinical practice, including olanzapine, quetiapine, risperidone and ziprasidone, or in advanced stages of development that are consistent with this model: asenapine, iloperidone, lurasidone, melperone, olanzapine, quetiapine, risperidone, sertindole, ziprasidone and zotepine and most importantly, none which are inconsistent with this hypothesis which means, that, unlike the so-called 'fast off' hypothesis of Kapur and Seeman (2000), it has not yet been falsified. This will be discussed in more detail subsequently.

Clozapine and related atypical antipsychotic drugs: 5-HT_{2A} and DA D₂ receptor antagonism

The 5-HT_{2A}/D₂ hypothesis

Utilizing in vitro binding data, Meltzer et al. (1989) concluded that clozapine and a group of other APDs, e.g. fluperlapine, melperone, amperozide, which produced minimal catalepsy or EPS at doses which are relevant to antipsychotic activity, i.e. atypical antipsychotics, had greater affinity for 5-HT_{2A} than D₂ receptors than did typical APDs, e.g. haloperidol, thiothixene, thioridazine, etc. and that D₁ receptor affinity did not contribute to differentiating atypical and typical APDs from one another. This 5-HT_{2A}/D₂ ratio hypothesis had a salutary influence on the subsequent development of APDs. In the 19 years since that hypothesis was brought forward, drugs that meet that test have almost entirely replaced the drugs which act predominantly through D₂ receptor blockade, even if they have some 5-HT_{2A} or α_2 blocking properties. In the case of chlorpromazine, for example the affinity for the D₂ receptor is greater than for the 5-HT_{2A} receptor:

2.0 and 3.2 nM, respectively (certified data using human cloned receptors; see the NIMH Psychopharmacology Drug Screening Program website, www.pdsp.med.unc.edu). In particular, the hypothesis that a relatively high affinity for the 5-HT_{2A} receptor compared to their affinities for the D₂ receptor was the basis for the difference between atypical and typical APDs contributed to the development of the newer antipsychotic agents such as asenapine, olanzapine, quetiapine, risperidone, sertindole and ziprasidone, all of which support the previously mentioned hypothesis of high affinity for 5-HT_{2A} and low affinity for D₂ receptors (Schotte et al., 1996). It was noted that other 5-HT receptors, or other non-serotonergic actions of these drugs, may also be important to some of their actions, including their antipsychotic effect and low potential for EPS (Meltzer et al., 1989). Among the 5-HT receptors, the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT₆ and 5-HT₇ receptors were suggested to be of additional interest (Meltzer et al., 1989; Meltzer and Nash, 1991). While some of the atypical APDs developed on the basis of the 5-HT_{2A}/D₂ hypothesis also have affinities for 5-HT_{2C}, 5-HT₃, 5-HT₆ or 5-HT₇ receptors in the same range as that for the 5-HT_{2A} receptor, this is not characteristic of all of these agents and, thus, it is not likely that affinities for these receptors are primary factors contributing to the low EPS profile of the entire class of agents (Roth et al., 1994; Meltzer and Fatemi, 1996; Schotte et al., 1996). Indeed, it has recently been reported that a 5-HT_{2C} agonist WAY-163909, shows antipsychotic properties in a variety of preclinical models (Marquis et al., 2007).

The 5-HT_{2A}/D₂ ratio hypothesis was recently challenged by Richtand et al. (2007). These authors found no significant relationship between the log of the 5-HT_{2A}/D₂ binding ratio and clinically effective dose for the atypical APDs, whereas a nearly significant relationship was reported for the typical APDs. There are at least six reasons for their findings. First, they studied only six compounds of the clozapine-class of drugs. Data are available for asenapine, iloperidone and perospirone, among others, that could have been included or would have been supplied by the NIMH Psychopharmacology Drug

Screening Program, which was the source of the data they used for the six compounds. Second, the clinical dose of clozapine utilized in their analysis is that for treatment resistant patients, which is at least twice that needed for non-treatment resistant patients whereas the doses used for the other 5-HT_{2A}/D₂ antagonists were for non-treatment resistant patients. Third, inclusion of amisulpride, a D₂/D₃ antagonist was inappropriate because the 5-HT_{2A}/D₂ hypothesis was never meant to include all drugs that are atypical or that the only means to achieve an antipsychotic action was via a dopaminergic mechanism. Fourth, and perhaps the largest contributor to their rejection of the 5-HT_{2A}/D₂ hypothesis, was using the affinity of aripiprazole, a partial DA agonist, as though aripiprazole was a direct acting D₂ receptor antagonist. Thus, they consider aripiprazole to be twice as potent as haloperidol as a D₂ receptor antagonist, which it most certainly is not. Removal of amisulpride and aripiprazole, and adjusting the dose of clozapine to 200–300 mg/day, completely negates their finding. The fifth reason, affecting only clozapine, is that its main metabolite, N-desmethylozapine (NDMC), is a unique agent which may contribute to the efficacy of clozapine through mechanism not shared by clozapine or any of the other 5-HT_{2A}/D₂ antagonists. The sixth reason for questioning the conclusion of Richtand et al. (2007) is pharmacokinetic. Differences in absorption and half-life can also affect the average clinical dose. The hypothesis has not been falsified by any agent to our knowledge which is a combined D₂ antagonist and 5-HT_{2A} inverse agonist with the correct ratio of the magnitude specified but has typical APD properties in man or laboratory animals.

Anatomy and function of 5-HT_{2A} receptors

5-HT_{2A} receptors are localized on the cell bodies of the ventral tegmental area (VTA) A10 DA and other non-dopaminergic VTA neurons (Doherty and Pickel, 2000). Jakab and Goldman-Rakic (1998) have demonstrated that 5-HT_{2A} receptors are also heavily located on most cortical pyramidal neurons, especially those above and below layers IV, as well as on many GABAergic interneurons

known to specialize in the perisomatic inhibition of pyramidal cells: large and medium-size parvalbumin- and calbindin-containing interneurons. Cortical 5-HT_{2A} receptors may play a crucial role in psychosis by virtue of their ability to modulate intracortical and cortical–subcortical glutamatergic neurotransmission. This could contribute to the ability of 5-HT_{2A} inverse agonists, including those atypical APDs which are potent in this regard, to attenuate some of the behavioural effects of PCP and ketamine. 5-HT_{2A} receptor inverse agonists such as ketanserin, ritanserin and M 100907 have been found to block MK-801-induced stereotypy and hyperlocomotion (Ninan and Kulkarni, 1998; Carlsson et al., 1999). Sub-chronic (seven day) PCP treatment of female rats was shown to impair cognition using a novel object recognition task (Grayson et al., 2007). Clozapine (1.0 and 5.0 mg/kg) and risperidone (0.2 mg/kg) but not haloperidol (0.05 mg/kg) significantly attenuated the PCP-induced impairment. Similarly, clozapine, olanzapine and risperidone, but not haloperidol or chlorpromazine attenuated the PCP-induced deficit in an operant conditioning paradigm (Abdul-Monim et al., 2006), suggesting that the reversal by the atypical drugs was more likely related to their shared 5-HT_{2A}/D₂ pharmacology rather than D₂ receptor blockade or some other pharmacologic features. Roth and colleagues have demonstrated lowered prefrontal cortical DA transmission and impairment of cognitive performance following repeated, intermittent administrations of PCP to monkeys. In monkeys withdrawn from repeated PCP treatment, using a dose regimen of clozapine that ameliorates the cognitive deficits produced by PCP, clozapine normalized DA turnover in the dorsolateral prefrontal cortex, prelimbic cortex and cingulate cortex, providing additional evidence the cognitive enhancing effect of clozapine may be related at least in part to restoring normal dopaminergic function in these and other brain regions.

5-HT_{2A} receptors and limbic and cortical DA efflux

Increased dopaminergic activity in the NAC, other mesolimbic, and possibly cortical regions as well,

may contribute to positive symptoms. As previously mentioned, clozapine and related atypical APDs, e.g. risperidone and olanzapine, have been shown to modestly improve selected areas of cognitive function in most patients with schizophrenia (Woodward et al., 2005). Atypical APDs have been shown to enhance DA efflux in the prefrontal cortex and hippocampus of rodents and monkeys (Moghaddam and Bunney, 1990; Youngren et al., 1994, 1999; Ichikawa et al., 2001, 2002a; Chung et al., 2004). The effect of these agents on cognition may be dependent, in part, upon their ability to increase the release of DA in PFC and hippocampus, which in turn has been correlated with their 5-HT_{2A}/D₂ ratio (Kuroki et al., 1999). We have found that the combination of a saturating dose of a 5-HT_{2A} inverse agonist/antagonist and a dose of D₂ antagonist which only partially blocks D₂ receptors leads to increased DA release in the frontal cortex (Bonaccorso et al., 2002; Liegeois et al., 2002; Huang et al., 2005). The 5-HT_{2A} receptor blockers do not potentiate D₂ receptor blockade when the dose of the latter is saturating (Liegeois et al., 2002). This is consistent with the hypothesis that at clinical doses, atypical APDs achieve partial blockade of D₂ receptors relative to 5-HT_{2A} receptor blockade. In addition, the atypical APDs enhance efflux of acetylcholine (ACh) in the PFC and hippocampus of rodents (Parada et al., 1997; Ichikawa et al., 2002b; Shirazi-Southall et al., 2002; Chung et al., 2004). It is possible that this effect may also contribute to their ability to improve cognition.

DOI, which is hallucinogenic in man, itself had no effect on basal DA release, but potentiated amphetamine-induced striatal DA release, and attenuated the ability of apomorphine, a direct acting D_{1/2/3} agonist, to decrease DA release in the striatum (Ichikawa and Meltzer, 1995). There is now considerable evidence from both behavioural and neurochemical studies involving NMDA antagonists such as PCP and MK-801 that 5-HT_{2A} receptors modulate activated but not basal mesolimbic DAergic function (Gleason and Shannon, 1997; De Deurwaerdere and Spampinato, 1999). Thus, stimulated DA release, e.g. with stress, may be increased in the forebrain terminal regions secondary to enhanced stimulation of 5-HT_{2A}

receptors. M100907 has been found to diminish the increase in DA efflux in the nucleus accumbens (NAC) produced by haloperidol (Liegeois et al., 2002) or S-sulpiride (Ichikawa et al., 2001). M100907 infused directly into the medial prefrontal cortex (mPFC) is resulted in a concentration-dependent blockade of K(+)-stimulated DA release and also blocked increases in DA release produced by the systemic administration of DOI. Thus, local 5-HT_{2A} antagonism has an inhibitory effect on stimulated DA release and suggest that cortical 5-HT_{2A} receptors potentiate the phasic release of mesocortical DA (Pehek et al., 2001). The local (in the mPFC) and systemic administration of DOI increased the firing rate and burst firing of DA neurons and DA release in the VTA and mPFC (Bortolozzi et al., 2005). The increase in VTA DA release was mimicked by the electrical stimulation of the mPFC. The effects of DOI were reversed by M100907. These results indicate that the activity of VTA DA neurons is under the excitatory control of mPFC 5-HT_{2A} receptors. Taken together, these data suggest that blockade of cortical 5-HT_{2A} receptors, by itself, may have antipsychotic action when dopaminergic activity is slightly to moderately increased. More studies are needed to define the ability of 5-HT_{2A} receptor antagonists to potentiate the action of low doses of D₂ receptor blockers in animal models as well as the clinic.

5-HT_{2A} Antagonism and animal models of psychosis

The hypothesis that the ratio of affinity for 5-HT_{2A} to D₂ receptors is a robust predictor of atypicality is supported by a variety of types of evidence (see Meltzer, 2002). M100907, or other selective 5-HT_{2A} receptor inverse agonists, either alone or in combination with selective antagonists of D₂ receptors, have been found to be effective in various animal models of psychosis. These include: (a) blockade of amphetamine-induced locomotor activity and inhibition of the firing of VTA dopaminergic neurons (Schmidt et al., 1995); (b) blockade of phencyclidine (PCP)- and dizocilpin (MK-801)-induced locomotor activity (Gleason and Shannon, 1997; Martin et al., 1997); (c) blockade of MK-801-induced prepulse inhibition (PPI) (Varty and Higgins, 1995); and

(d) antipsychotic-like activity in the paw test (Prinssen et al., 1994), among others. Of particular interest is the report of Wadenberg et al. (1998) that the combination of an ED₅₀ dose of raclopride, a D₂ receptor antagonist and M100907, but not M100907 alone, was effective in blocking the conditioned avoidance response. They concluded that 5-HT_{2A} antagonism alone could not achieve an antipsychotic action but was able to potentiate blockade of D₂ receptors to achieve such an effect. This corresponds much more closely to the apparent clinical situation than does the models above where M100907 alone was effective, e.g. blockade of PCP- or MK-801-induced locomotor activity. Pimavanserin, another 5-HT_{2A} inverse agonist, has been shown to potentiate the effect of haloperidol and risperidone to enhance cortical DA efflux in rats (Huang et al., 2005). Gardell et al. (2007) have recently reported that ACP-103 reduced the dose of haloperidol and risperidone required for activity in amphetamine- or MK-801-induced hyperactivity models and suppressed haloperidol-induced hyperprolactinemia in mice. NRA0045, which has potent 5-HT_{2A}, D₄ and α_1 but negligible D₂ or D₃ receptor blockade, has been found to have atypical antipsychotic properties in rodents (Okuyama et al., 1997a, b). Ishikane et al. (1997) reported that M 100907 is able to block haloperidol-induced catalepsy only at low doses of haloperidol. Weiner et al. (2001) profiled APDs for functional activity at 33 of the 36 known human monoaminergic G-protein coupled receptors using the mammalian cell-based functional assay Receptor Selection and Amplification Technology (R-SAT). Competitive antagonism of D₂ receptors and inverse agonism of 5-HT_{2A} receptors was nearly uniform throughout this class, with typical agents demonstrating low 5HT_{2A}/D₂ ratios, and atypical agents demonstrating high ratios.

Olanzapine and clozapine, two atypical APDs, more potently reversed the amphetamine-induced inhibition of A10 DA neurons compared to the substantia nigra pars compacta (SNc) A9 DA neurons (Olijslagers et al., 2005). These authors also reported that risperidone (0.03 and 0.1 μ M) reversed amphetamine-induced inhibition of firing activity of both A9 and A10 DA neurons but with

no difference in potency. Risperidone has a higher affinity for D₂ relative to 5-HT_{2A} receptors than either clozapine or olanzapine. The DA D₂ receptor antagonist (–)sulpiride (0.05 and 1 μ M) reversed the amphetamine (10 μ M)-induced inhibition of firing activity in A9 and A10 neurons. Moreover, the selective 5-HT_{2A} receptor antagonist M100907 (0.05 μ M), strongly enhanced the reversal of amphetamine-induced inhibition by (–)sulpiride in A10, but its effectiveness to enhance the effect of sulpiride was much less in A9 DA neurons. They suggested that 5-HT_{2A}, in combination with DA D₂ receptor antagonism, may play a role in atypical APD differential effects on nigrostriatal and mesocorticolimbic DAergic activity, leading to some of the clinical differences between typical and atypical APDs.

The ability of drugs to reverse PCP-induced disruption of PPI is correlated to their affinity for 5-HT_{2A}, not D₂ receptors (Yamada et al., 1999). Interestingly, the 5-HT_{2A} antagonist, M100907 and the α_1 -adrenergic antagonist, prazosin, are also effective to reverse PPI deficits induced by NMDA receptor antagonists (Geyer et al., 2001) but the α_2 antagonist RX821002, the M1/M4 muscarinic antagonist pirenzepine or the GABA-A antagonist picrotoxin had no effect on basal or PCP-impaired PPI in mice (Bakshi and Geyer, 1997). It has been suggested that the hallucinogenic action of 5-HT_{2A} agonists such as the hallucinogen DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) is mediated by mGluR 2/3 receptor-induced glutamate release, as the head shakes induced by DOI are blocked by the mGluR 2/3 agonist LY354740 and potentiated by the mGluR 2/3 antagonist LY341495 (Gewirtz and Marek, 2000).

Postmortem and PET studies of 5-HT_{2A} receptor density

Numerous studies have examined the density of 5-HT_{2A} receptors in various cortical regions of patients with schizophrenia with decreased (Arora and Meltzer, 1991; Burnet et al., 1996; Dean et al., 1999), increased (Joyce et al., 1993) or normal levels reported. It is well established that some typical and atypical APDs can decrease the density of 5-HT_{2A} receptors (Matsubara and Meltzer,

1989), so the postmortem results noted above may be related to drug treatment. Importantly, a PET study in never-medicated patients with schizophrenia reported a decrease in cortical 5-HT_{2A} receptor density (Ngan et al., 2000) whereas studies which included medicated patients found no decrease in 5-HT_{2A} receptors in the cortex of patients with schizophrenia (Trichard et al., 1998a, b; Verhoeff et al., 2000). Hurlmann et al. (2008) quantified the spatial distribution of 5-HT_{2A} receptor binding potential using [(18F)]altanserin positron emission in never-medicated individual who were considered to be in six early and eight late prodromal states of schizophrenia relative to 21 healthy controls. They found decreases in binding potential which increased with extent of risk as well as greater decreases in subjects who subsequently converted to first-episode psychosis ($n = 5$) compared to non-converters ($n = 9$). Progressive reduction of cortical 5-HT_{2A} receptor density was proposed as a surrogate biological measure of increased risk for schizophrenia, irrespective of conversion. Diminished density of 5-HT_{2A} receptors in schizophrenia could be related to excessive stimulation of 5-HT_{2A} receptors by endogenous 5-HT.

PET studies utilizing ¹¹C-raclopride as the ligand led to the suggestion that occupancy of at least 60% of striatal D₂ receptors is required for an antipsychotic action by typical APDs and, at least some atypical APDs, including risperidone and olanzapine, and that $\geq 80\%$ D₂ receptor occupancy is associated with EPS (Farde et al., 1992; Kapur et al., 1995, 2000; Talvik et al., 2001; Mamo et al., 2004; see Miyamoto et al., 2005, for review). However, we and others have shown, that clozapine and quetiapine are effective at lower occupancies of D₂ receptors than typical and other atypical APDs, e.g. risperidone, olanzapine and ziprasidone, implying some mechanism other than D₂ receptor blockade is contributing to their low side effect profile and perhaps their efficacy, as well. Using [¹⁸F]fallypride PET studies in patients treated with olanzapine, quetiapine or clozapine, we have shown that occupancy of striatal receptors in patients responding to these drugs is often less than 60% (Kessler et al., 2005, 2006). Clozapine, the most effective of these agents, was found to

produce the lowest occupancy of DA D₂ receptors in the midbrain DA neurons which give rise to the three main DA ascending DA pathways. Grunder et al. (2006) also found lesser occupancy of striatal D₂ receptors with clozapine and even lower extrastriatal than striatal binding. It was concluded that extrastriatal D₂/D₃ receptor binding may be more relevant to the antipsychotic action of clozapine than striatal D₂/D₃ receptor binding. The Kapur group itself has shown that that D₂ receptor occupancy of less than 60% with risperidone and quetiapine still leads to antipsychotic efficacy (Mamo et al., 2008a, b). This is an important finding because the 60% occupancy theory has been relied upon extensively to identify minimal dosages in the development of new APDs related to the 5-HT_{2A}/D₂ hypothesis.

Higher occupancy of 5-HT_{2A} receptors than D₂ receptors have been reported in volunteers or patients treated with atypical APDs (Fischman et al., 1996; Kapur et al., 2005; Kessler et al., 2005), whereas typical APDs such as chlorpromazine, while they may strongly block 5-HT_{2A} receptors, cause more extensive blockade of limbic/striatal D₂ receptors (Trichard et al., 1998a, b). This is consistent with the hypothesis that it is the relative amount of 5-HT_{2A} and D₂ receptor blockade that is important for an atypical APD profile.

Clinical trials testing 5-HT_{2A}/D₂ ratio hypothesis and antipsychotic potential of 5-HT_{2A} antagonism, alone, or in combination with D₂ antagonists

The bell-shaped dose response curve of risperidone, with higher doses being less effective than lower doses (Marder, 1994), suggests that excessive D₂ receptor antagonism may diminish some of the beneficial effects of 5-HT_{2A} receptor blockade (Meltzer and Fatemi, 1996). This is supported by two recent studies in which risperidone was added to clozapine treatment of partial responders in placebo-controlled randomized clinical trials. Placebo was generally superior to risperidone in a number of outcome measures in both studies (Anil Yagcioglu et al., 2005; Honer et al., 2006). Nevertheless, it is noteworthy that there are uncontrolled reports to the contrary involving

D₂ receptor blockers which are sometimes positive (see [Stahl and Grady, 2004](#), for review). The positive case reports may reflect individuals who do, in fact, require more extensive D₂ receptor blockade or who are resistant for pharmacodynamic or pharmacokinetic reasons, to the action of 5-HT_{2A} receptor blockade.

The highly selective 5-HT_{2A} inverse agonist M100907, formerly MDL 100907, was shown in a randomized, double-blind study to be more effective than placebo for treating positive and negative symptoms in hospitalized schizophrenic patients ([Shipley, 1998](#)). However, because it was less effective than haloperidol, no further development of its utility in schizophrenia followed. The 5-HT_{2A/2C} selective antagonist SR 46349B ([Rinaldi-Carmona et al., 1993](#)) was nearly as effective as haloperidol in treating patients with schizophrenia in a randomized double blind clinical trial ([Meltzer et al., 2004](#)). Low doses of three atypical APDs, clozapine, quetiapine and melperone ([Barbato et al., 1996](#)), are effective and tolerable in the treatment of L-DOPA psychosis. We have proposed that this is due to their 5-HT_{2A} receptor blockade, sparing D₂ receptors ([Meltzer, 1995](#)). The selective 5-HT_{2A/2C} inverse agonist, pimavanserin, (N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy)phenylmethyl)carbamide (2*R*,3*R*)-dihydroxy butanedioate (2:1); ACP103), ([Vanover et al., 2006](#); [Li et al., 2005a, b](#)) was recently found to be able to reduce delusions but not hallucinations, without interfering with motor function ([Meltzer et al., in preparation](#)) and to potentiate the ability of subtherapeutic doses of risperidone, 2 mg/day, to improve psychosis in acutely psychotic patients with schizophrenia ([Meltzer et al., in preparation](#)). The combination of low dose risperidone and pimavanserin was significantly more effective than risperidone 6 mg/day in improving Positive and Negative Syndrome Scale (PANSS). Total and positive symptom subscale rating at 14 days but not different at later times in this randomized, double-blind multicenter trial. Replication of this important finding is needed. Should this strategy prove effective, it will provide strong support for the 5-HT_{2A}/D₂ hypothesis ([Meltzer et al., 1989](#)).

Pharmacogenomic evidence for the role of 5-HT_{2A} receptors in atypical antipsychotic drug action

The role of 5-HT_{2A} receptor blockade in the action of clozapine and possibly other drugs with potent 5-HT_{2A} affinities is supported by the evidence that the His452Tyr allele of the 5-HT_{2A} receptor, which is present in 10–12% of the population, is associated with a higher frequency of poor response to clozapine ([Masellis et al., 1998](#)). In addition, the T102C single nucleotide and promoter polymorphisms have also been reported to be related to response to clozapine ([Arranz et al., 1995, 1998a, b](#)).

Prolactin secretion and 5-HT_{2A}/D₂ antagonists

Prolactin secretion is most strongly influenced by inhibitory D₂ receptors in the anterior pituitary gland. Typical neuroleptic drugs increase the release of prolactin in vivo and in vitro by blocking these receptors. Atypical APDs produce minimal increases in prolactin secretion in man when given acutely or chronically. They do produce transient increases in prolactin in rodents which we have shown is the result of their ability to increase the release of DA into the pituitary portal circulation and thus overcome whatever D₂ receptor blockade they produce at the level of the pituitary gland ([Gudelsky et al., 1987](#)). Risperidone is only the atypical APD which causes marked increases in prolactin secretion. The explanation of its differences from other atypicals which have similar D₂ affinities, such as olanzapine, has not yet been obtained. Conceivably, it is unable to enhance the release of DA from the tuberoinfundibular DA neurons.

Preclinical and clinical studies with 5-HT_{2A} hallucinogens

The 5-HT_{2A} and 5-HT_{1A} agonist, psilocybin, has been reported to impair cognitive function in normal volunteers ([Hasler et al., 2004](#)). 5-HT_{2A} receptors have been implicated in the genesis of, as well as the treatment of, psychosis, negative symptoms, mood disturbance and EPS. There has been a long controversy as to whether the

hallucinogenic effects of indole hallucinogens are mediated by stimulation of 5-HT_{2A} or 5-HT_{2C} receptors, or both (Fiorella et al., 1995). Recent studies from the Sanders–Bush laboratory have suggested it is the 5-HT_{2A} receptor which is more important (Gresch et al., 2002). The hallucinogenic drug lysergic acid diethylamide (LSD) produced a five to eightfold increase in Fos-like immunoreactivity in rat mPFC, anterior cingulate cortex and central nucleus of amygdala. However, in dorsal striatum and NAC no increase in Fos-like immunoreactivity was observed. Pre-treatment with the 5-HT_{2A} receptor antagonist MDL 100907 completely blocked LSD-induced Fos-like immunoreactivity in mPFC and anterior cingulate cortex, but only partially blocked LSD-induced Fos-like immunoreactivity in amygdala. Vollenweider and colleagues have compared the 5-HT_{2A} hallucinogenic agonist psilocybin and concluded that it provides a better model for psychosis than does ketamine, an NMDA receptor non-competitive antagonist (Vollenweider et al., 2007). This is a very important finding because the field has embraced the glutamate hypothesis of schizophrenia largely on the basis of the NMDA receptor antagonist, phencyclidine, being a better model for both the psychosis and cognitive impairment of schizophrenia (Coyle, 2006).

Partial DA agonist atypical antipsychotics with contributory 5-HT actions

Most APDs, both typical and atypical, which have affinity for D₂ and D₃ receptors are inverse agonists at both receptors (Burstein et al., 2005). However, some are partial D₂/D₃ agonists, e.g. aripiprazole (Swainston Harrison and Perry, 2004), bifeprunox (Newman-Tancredi et al., 2007) and NDMC, the major metabolite of clozapine (Bruins Slot et al., 2005; Burstein et al., 2005). Partial agonists, by definition, produce lesser activation of the D₂/D₃ receptors than the full agonist and as such, will produce smaller functional responses than the endogenous neurotransmitter (Tamminga, 2002). Partial agonists are particularly more potent as agonists when there is high receptor reserve. Thus, DA partial agonists are more likely to stimulate DA autoreceptors

than post-synaptic DA receptors. Because of this, they diminish dopaminergic stimulation through a dual mechanism: suppression of the release of DA by stimulating autoreceptors, and blocking the response to the full agonist at post-synaptic receptors.

There have been many attempts to develop DA autoreceptor agonists for the treatment of schizophrenia but all failed until recently, when DA partial agonists which also had 5-HT_{2A} inverse agonist or 5-HT_{1A} partial agonist properties, or both, were tested and were found to be effective and tolerable. Thus, aripiprazole, an approved atypical APD (Swainston Harrison and Perry, 2004), is both a 5-HT_{2A} inverse agonist and 5-HT_{1A} partial agonist (Burris et al., 2002; Li et al., 2004). Bifeprunox is a partial agonist at both the D₂ and 5-HT_{1A} receptors only, with no 5-HT_{2A} antagonist properties (Newman-Tancredi et al., 2007). It is in phase III testing as an APD. Both drugs produce minimal EPS at clinically effective doses. As agonists, both can suppress serum prolactin levels. Aripiprazole, but not bifeprunox, has been reported to reverse deficits in social interaction in rats produced by the NMDA receptor antagonist, phencyclidine (Bruins Slot et al., 2005).

We have shown that the ability of bifeprunox to enhance cortical DA efflux, while limited, is related to its 5-HT_{1A} partial agonist properties as it is blocked by the 5-HT_{1A} antagonist, WAY-100635 (Li et al., 2004). Similar data has been obtained for bifeprunox in our laboratory. These results have been replicated (Zocchi et al., 2005). While both compounds are effective to treat psychosis, aripiprazole may be the more effective of the two compounds based upon how well they have performed in trials with risperidone or olanzapine as the active comparator. However, until they have been tested in sufficient numbers of head to head trials this cannot be concluded to be the fact. If this inference is supported by further study, it would suggest that 5-HT_{2A} receptor antagonism is more important for treating psychosis, and that 5-HT_{1A} partial agonism, at least when combined with D₂ partial agonism, does not completely replace the beneficial effects of 5-HT_{2A} receptor blockade. Further study is needed to compare

aripiprazole and bifeprunox with those atypicals which are D₂ antagonists, e.g. risperidone, before concluding that D₂ partial agonism is a less efficacious means to reducing excessive limbic dopaminergic function than is D₂ receptor blockade.

Neuroplasticity, neuroprotection and serotonin_{2A} receptors

Brain-derived neurotrophic factor (BDNF) regulates survival, differentiation, synaptic strength and neuronal morphology in the cerebral cortex and hippocampus in frontal and other cortical areas, while decreasing its expression in the dentate gyrus granule cell layer. The BDNF-gene Val66Met polymorphism has been reported to be associated with schizophrenia and response to clozapine (Hong et al., 2003). It has recently been demonstrated that stimulation of 5-HT_{2A} receptors by the 5-HT_{2A/2C} agonist DOI and stress increases the expression of BDNF in cortex and hippocampus (Vaidya et al., 1997). These effects were blocked by the 5-HT_{2A} antagonists M100907 and ritanserin, respectively, which by themselves did not have any effect in these regions. Electrophysiological studies with slices from rats following chronic treatment with clozapine or haloperidol showed that chronic clozapine but not haloperidol treatment resulted in an attenuation of the effect of the activation of 5-HT_{2A} receptors, without changing response to 5-HT_{1A} and 5-HT₄ receptor activation. These data are consistent with the hypothesis that chronic clozapine selectively attenuates 5-HT-mediated excitation in neuronal circuitry of the frontal cortex while leaving 5-HT-mediated inhibition intact (Zahorodna et al., 2004). Clozapine and olanzapine up-regulated BDNF mRNA expression in CA1, CA3 and dentate gyrus regions of the rat hippocampus whereas haloperidol (1 mg/kg) down-regulated BDNF mRNA expression in both CA1 (P<0.05) and dentate gyrus (P<0.01) regions (Bai et al., 2003). Neither acute nor chronic clozapine treatment significantly affected the expression of BDNF mRNA in various brain areas. However, the NMDA antagonist MK-801 (5 mg/kg; 4 h) significantly increased BDNF mRNA in the

entorhinal cortex, an effect which was attenuated by pre-treatment with clozapine and haloperidol (Linden et al., 2000). These data suggest that clozapine-like atypical APDs, via their 5-HT_{2A} antagonism, might modulate the activity of BDNF and possibly other growth factors.

Although the cellular signalling pathways required for inducing these actions have not yet been determined, roles for the neuroprotective extracellular-regulated kinase (ERK), mitogen-activated protein (MAP) kinase and Akt (protein kinase B) pathways have been suggested. Agonists for 5-HT_{2A} as well as 5-HT_{1A} and 5-HT₇ receptors have been found to activate ERK and Akt in transfected PC12 cells, two neuroprotective pathways. Evidence supporting the role of 5-HT_{2A} receptors in neuroprotection due to atypical APDs was recently reported by Ukai et al. (2004). The neurotoxicity of risperidone and haloperidol were compared in primary cultured rat cortical neurons. Haloperidol induced significantly more apoptotic injury to the neurons compared to risperidone. This was related to a greater reduction of phosphorylation of Akt, and activated caspase-3 by haloperidol which was attenuated by the 5-HT_{2A} antagonist ketanserin and the D₂ agonist bromocriptine. BDNF had a similar neurochemical and protective effect (Ukai et al., 2004).

Conclusions: 5-HT_{2A} receptors and in vivo actions of antipsychotic drugs related to serotonin and dopamine

As reviewed above, the ability of atypical APDs related to clozapine, i.e. olanzapine, risperidone, quetiapine, ziprasidone, iloperidone, asenapine, etc. have the clear ability to enhance DA efflux in the cortex and hippocampus while producing significantly smaller effects in the limbic system. The typical APDs have the opposite pattern. The difference between these drugs is related to the 5-HT_{2A} receptor antagonism of the atypical APDs as the combination of a 5-HT_{2A} inverse agonist and subthreshold doses of the atypicals or a typical agent, e.g. haloperidol, can produce similar effects. There is also evidence from emerging studies of the combination of selective 5-HT_{2A} antagonists and

subthreshold doses of atypicals that 5-HT_{2A} antagonism contributes to the ability of these agents to improve cognitive dysfunction in chronically PCP treated rodents and monkeys. The latter effects of the atypical agents also appear to be due, in part to DA efflux. Clinical trials with selective 5-HT_{2A} inverse agonists are limited but what there is, is supportive of the view that presumptively DA-mediated psychoses in schizophrenia and in Parkinson's disease respond to 5-HT_{2A} receptor blockade.

Contributions of other 5-HT receptors to atypical antipsychotic drug efficacy and side effects

The role of the 5-HT_{1A} receptor in antipsychotic drug action: 5-HT_{1A} and 5-HT_{2A} interactions

5-HT_{1A} receptors are located pre- and post-synaptically. The pre-synaptic 5-HT_{1A} receptor is an autoreceptor located on cell bodies of raphe neurons; stimulation leads to inhibition of firing of 5-HT neurons. Stimulation of post-synaptic 5-HT_{1A} receptors leads to hyperpolarization of pyramidal neurons, opposite to the effect of stimulation of 5-HT_{2A} receptors. Approximately 60% of rat mPFC glutamatergic cells were found to have 5-HT_{1A} mRNA as did approximately 25% of GAD-expressing cells (Santana et al., 2004). 5-HT_{1A} receptor agonists have effects similar to 5-HT_{2A} receptor inverse agonists in a variety of functions (Darmani et al., 1990; Meltzer and Maes, 1995). A few examples will be given. For example, DOI injected bilaterally into the rat medial PFC elicited a dose-dependent head twitch response. This effect is inhibited by the 5-HT_{1A} agonist 8-OH-DPAT as well as the 5-HT_{2A} inverse agonists M100907 and ketanserin. Ahlenius (1989) first suggested that stimulation of 5-HT_{1A} receptors might produce an antipsychotic-like action on the basis of behavioural studies in animals using the direct 5-HT_{1A} agonist 8-OH-DPAT. Subsequent studies demonstrated that 8-OH-DPAT enhanced the antipsychotic-like effect of the D₂/D₃ antagonist raclopride (Wadenberg and Ahlenius, 1991) and of haloperidol (Prinssen et al., 1996), and antagonized the catalepsy

induced by the D₁ agonist SCH23390 in rats (Wadenberg, 1992). The beneficial effect of 5-HT_{1A} agonists appears to be mediated by inhibition of median raphe serotonergic neurons (Wadenberg and Hillegaart, 1995). Ichikawa and Meltzer (2000) demonstrated that 8-OH-DPAT inhibited the ability of clozapine and low dose risperidone, but not haloperidol, to increase extracellular DA levels in the NAC and the striatum of conscious rats. The effect in the NAC would be expected to enhance the antipsychotic effect of these agents by reducing dopaminergic activity in this region which is believed to participate in the generation of psychotic symptoms. Several atypical APDs are partial agonist at the 5-HT_{1A} receptor including aripiprazole, bifeprunox, clozapine, ziprasidone, quetiapine and tirosiprone. Their affinities for the 5-HT_{1A} receptor were similar to their affinities at the human D₂ receptor (Newman-Tancredi et al., 1998).

The ability of atypical APDs to increase DA efflux in the rat PFC and hippocampus is blocked, in part, by WAY-100635, a 5-HT_{1A} antagonist (Rollema et al., 1997; Ichikawa et al., 2001, 2002a; Chung et al., 2004). This effect of 5-HT_{1A} receptors appears to be mediated by 5-HT_{1A} receptors in the PFC as it is blocked by local injection of a 5-HT_{1A} antagonist in the mPFC or by transaction of cortical connections to the VTA (Diaz-Mataix et al., 2005). The atypical APD perospirone, which has D₂ and both 5-HT_{2A} antagonist and 5-HT_{1A} agonist properties was shown to reverse the cognitive deficit in mice produced by repeated administration of PCP. Subsequent sub-chronic administration of perospirone reversed the effects of PCP in a dose-dependent manner. Co-administration of the 5-HT_{1A} selective antagonist WAY 100635 significantly antagonized the effect of perospirone (Hagiwara et al., 2007).

These findings suggest that the combination of D₂ antagonism and 5-HT_{1A} agonism should produce an atypical antipsychotic agent. S16924 is an example of such a compound and it has atypical properties very similar to those of clozapine in a variety of animal models (Millan et al., 1998). The 5-HT_{1A} partial agonist S15535 enhanced the release of ACh in rat mPFC

(Millan et al., 2004). However, there is little evidence so far that the increase in ACh efflux in mPFC or hippocampus produced by atypical APDs is related to their direct or indirect 5-HT_{1A} agonism (Ichikawa et al., 2002b; Chung et al., 2004). Bruins Slot et al. (2005) have suggested that the balance of activity at D₂ and 5-HT_{1A} receptors is important for the ability of APDs to reverse the deficit in social interaction, a putative model of negative symptoms, in rats.

The role of the 5-HT_{2C} receptor in antipsychotic drug action: 5-HT_{2A} and 5-HT_{2C} interactions

There has been extensive consideration given to the role of 5-HT_{2C} receptors in the action of atypical APDs. The 5-HT_{2C} receptor is found throughout the CNS, including the VTA and the NAC (Pazos et al., 1987). The 5-HT_{2C} receptor is constitutively active, meaning it is activated even in the absence of agonist (Barker et al., 1994; De Deurwaerdere et al., 2004). The ser23cys single nucleotide polymorphism of the HTR_{2C} receptor gene has been reported to be predictive of response to clozapine (Sodhi et al., 1995). The HTR_{2C} receptor gene undergoes extensive RNA editing in brain, which leaves open the possibility that there are multiple forms of the receptor in brain (Niswender et al., 1999). This, in turn, suggests the HTR_{2C} gene may be very important to epigenetic events which may influence the course of schizophrenia and response to treatment (Reynolds et al., 2005; Sodhi et al., 2005). Because of the development of specific 5-HT_{2C} agonists, inverse agonists and antagonists, it has been possible to obtain evidence for a tonic inhibitory action of 5-HT_{2C} receptors on the burst firing of mesolimbic and mesocortical dopaminergic neurons. Thus, the firing rate of VTA DA neurons is inhibited or increased by 5-HT_{2C} agonists or antagonists, respectively. This is consistent with microdialysis studies which show that 5-HT_{2C} antagonists increase extracellular concentrations of DA in the NAC and medial PFC (Millan et al., 1998; De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1999). These studies establish that the 5-HT_{2C} receptor is most important for regulation of tonic DA release. We have recently reported

that the combination of the 5-HT_{2C} neutral antagonist SB242984, 1.0 mg/kg, and low or high dose haloperidol, enhanced the release of cortical and NAC DA in rats using microdialysis (Li et al., 2005a, b). SB242084, 0.2 mg/kg, produced significant increase in DA efflux in the NAC, not the mPFC. The extent of both 5-HT_{2C} and 5-HT_{2A} receptor blockades, in relation to D₂ receptor blockade, may be a key factor in the relative ability of atypical APDs to preferentially increase cortical DA efflux compared to NAC DA efflux (Li et al., 2005a, b).

Early studies found no significant differences between groups of atypical APDs and typical neuroleptics with regard to the affinity for 5-HT_{2C} receptor or the difference between 5-HT_{2C} and D₂ affinities have been reported (Roth et al., 1992, 1994; Schotte et al., 1996). Several typical APDs (chlorpromazine, thioridazine, spiperone, thiothixene) displayed neutral 5-HT_{2C} antagonist activity by reversing clozapine-induced inverse agonism. A large group of atypical APDs (sertindole, clozapine, olanzapine, ziprasidone, risperidone, zotepine, tiospirone, fluperlapine, tenilapine) show potent inverse agonist activity at rat and human 5-HT_{2C} receptors (Herrick-Davis et al., 2000). Chronic sertindole but not clozapine, down-regulated cortical 5-HT_{2C} receptors (Hietala et al., 2001). These data are consistent with the concept that 5-HT_{2C} receptor blockade of constitutive and stimulated 5-HT_{2C} receptor activity may play a role in the action of some atypical APDs.

Of the approved atypical APDs, some have equivalent affinities for the 5-HT_{2A} and 5-HT_{2C} receptors (clozapine, olanzapine, sertindole) while others are more selective for the 5-HT_{2A} receptor (risperidone, quetiapine, ziprasidone). This difference roughly corresponds with potential to produce weight gain in that clozapine and olanzapine cause the greatest weight gain, quetiapine intermediate, and aripiprazole, bifeprunox, risperidone and ziprasidone the least. The 759T polymorphism in the promoter region of the 5-HT_{2C} receptor gene has been found to be associated with clozapine-induced weight gain, response to clozapine and tardive dyskinesia (Reynolds et al., 2005). 5-HT_{2C} receptor antagonism appears to have useful effects on certain types of memory impairment. Thus, SB-200646 antagonized memory impairment

due to some 5-HT_{2A/2C} agonists and dizocilpine but not scopolamine (Meneses, 2002).

An important new development with regard to the 5-HT_{2C} receptor and antipsychotic action is the report that the behavioural profile of the 5-HT_{2C} selective receptor agonist WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole] is similar to that of an atypical antipsychotic. Consistent with an antipsychotic action, WAY-163909 decreased apomorphine-induced climbing and blocked conditioned avoidance responding, with no significant catalepsy. Like other atypical APDs, in vivo electrophysiological recordings showed a decrease in the number of spontaneously firing DA neurons in the VTA but not in the SNc with both acute and chronic (21-day) administration. WAY-163909 also more potently reduced PCP-induced locomotor activity compared with D-amphetamine, with no effect on spontaneous activity. In this regard, it is similar to 5-HT_{2A} antagonists, as previously discussed. Finally, suggesting the ability to improve cognition, WAY-163909 (1.7–17 mg/kg i.p.) reversed MK-801 and DOI-disrupted PPI and improved PPI in DBA/2N mice. We demonstrated that WAY-163909 produced a dose-dependent decrease in DA efflux in the NAC, which may contribute to its antipsychotic action. Moreover, it produced a significant increase in cortical ACh efflux, as do atypical APDs, as well as a small increase in cortical DA efflux. In this regard, it differs from other 5-HT_{2C} agonists which have been shown to decrease cortical ACh efflux. It will be of particular interest to test the effects of this agent with regard to its ability to improve cognition in patients with psychosis. WAY-163909 and other 5-HT_{2C} agonists might be most useful when linked to pharmacogenomic testing of patients to determine if specific types of 5-HT_{2C} receptor mutations or editing influences efficacy. It may also have some antidepressant and appetite suppressant effects (Dunlop et al., 2006).

Possible role of 5-HT₆ receptor antagonism in atypical antipsychotic drug action

Recent interest in the 5-HT₆ receptor in relation to the action of APDs has been based, in part, on the

finding that some APDs, including the atypical APDs clozapine, olanzapine and sertindole, are relatively potent 5-HT₆ receptor antagonists (Monsma et al., 1993; Roth et al., 1994; Kohen et al., 1996; Arnt and Skarsfeldt, 1998; Boess et al., 1998). Regional analysis of receptor binding and the expression of 5-HT₆ receptor mRNA revealed that the highest levels are found in the striatum, olfactory tubercle, NAC, cerebral cortex and subfields of the hippocampus (Monsma et al., 1993; Ruat et al., 1993; Gerard et al., 1996, 1997; Hamon et al., 1999). Together, these data on expression and localization suggests possible involvement of 5-HT₆ receptors in cognitive, affective and motor function.

It has been reported that clozapine, the prototypical APD, but not haloperidol, significantly decreased 5-HT₆ receptor expression in all subfields of the hippocampus (Frederick and Meador-Woodruff, 1999). As clozapine has a greater effect than haloperidol to improve some aspects of cognition in schizophrenia (Hagger et al., 1993), it was suggested that downregulation of this receptor in the hippocampus might contribute to the ability of clozapine to enhance cognition (Frederick and Meador-Woodruff, 1999). This hypothesis is supported by a series of behavioural studies which demonstrate that the 5-HT₆ receptors may be involved in learning and memory (Rogers et al., 1999; Huber et al., 2000; Meneses, 2001; Rogers and Hagan, 2001; Stean et al., 2002; Woolley et al., 2003; Lieben et al., 2005). These data suggests that 5-HT₆ receptor stimulation may interfere with learning and long term memory function and the potential role for 5-HT₆ receptor antagonists for the enhancement of cognition in patients with schizophrenia. The high affinity of some atypical APDs for 5-HT₆ receptors, and the localization of 5-HT₆ receptor in limbic and cortical regions of the brain, suggest that 5-HT₆ receptors also play a role in the mechanism of action and pathophysiology of some aspects of schizophrenia. Minabe et al. (2004) reported that high but not low doses of the selective 5-HT₆ receptor antagonist SB-271046 given acutely suppressed the firing rate of VTA DA neurons. Chronic administration altered the pattern of firing of these neurons which are highly

relevant to schizophrenia in a manner which differed from that of either typical or atypical APDs. It was suggested that clinical study of this type of agent would be of interest to determine its role, if any, in the treatment of schizophrenia. There has been only limited study of the role of the 5-HT₆ receptors in the modulation of DA or ACh release, which might be relevant to their ability to improve cognition, and the results have been inconsistent. 5-HT₆ receptor antagonists have been reported to increase extracellular ACh efflux in the cortex or hippocampus in two studies (Sleight et al., 1999; Riemer et al., 2003). However, another 5-HT₆ receptor antagonist Ro 04-6790 failed to do so (Shirazi-Southall et al., 2002).

The 5-HT₆ receptor antagonist SB-271046, which has been shown to be effective in enhancing cognitive function in models of learning and memory (Rogers et al., 1999; Rogers and Hagan, 2001), had no effect on DA efflux in either the frontal cortex or dorsal hippocampus (Dawson et al., 2001). However, a recent study reported that SB-271046 produced a significant increase in DA release in the rat mPFC (Lacroix et al., 2004). Pouzet et al. (2002) found that SB-271046 dose-dependently inhibited D-amphetamine-induced suppression of PPI, consistent with an antipsychotic action. However, Leng et al. (2003) found no effect of two other 5-HT₆ antagonists in latent inhibition (LI) or PPI models in which clozapine was active. The 5-HT₆ receptor antagonist, N-[4-methoxy-3-(4-methyl-1-piperazinyl)-phenyl]-5-chloro-3-methylbenzo-thiophene-2-yl sulphonamide monohydrochloride (SB 258510A), has been reported to produce greater potentiation of amphetamine-induced increase in extracellular DA release in the medial PFC than the NAC (Frantz et al., 2002). Another 5-HT₆ receptor antagonist, SB 271046, potentiated amphetamine-induced DA release in the striatum (Dawson et al., 2003). Li et al. (submitted) found that SB-399885, 3 and 10 mg/kg, a selective 5-HT₆ receptor antagonist had no effect on cortical DA efflux but slightly increased hippocampal DA efflux in freely moving rats. However, SB-399885, 3 mg/kg, significantly potentiated the ability of haloperidol, a D₂ receptor antagonist, at a dose of 0.1 mg/kg, to

increase DA release in the hippocampus but not the mPFC. SB399885 also potentiated risperidone, 1.0 mg/kg-induced DA efflux in both hippocampus and mPFC. These results suggest that the combined blockade of 5-HT₆ and D₂ receptor may contribute to the potentiation of haloperidol- or risperidone-induced DA efflux in the mPFC and hippocampus. In addition, other microdialysis studies suggest that the 5-HT₆ receptor may interact with DA mechanisms in the rat medial PFC as antisense oligonucleotides partially antagonized the fluoxetine-induced cortical DA release (Matsumoto et al., 1999). Taken together these data suggest that 5-HT₆ receptors may have modulatory influence on DA efflux in the mPFC and hippocampus, and, hence, may contribute to some of the clinical benefits of atypical APDs. 5-HT₆ receptor antagonists may also be useful as augmenting agents to improve cognitive dysfunction in schizophrenia. There is, however, insufficient evidence to conclude that they will be first line treatments for the psychosis of schizophrenia.

The role of 5-HT release in atypical antipsychotic drug action

The antagonism of multiple 5-HT receptors by clozapine would be expected to enhance the release of 5-HT by feedback mechanisms. Ichikawa et al. (1998) reported that clozapine (20 mg/kg) and risperidone (1 mg/kg) but not olanzapine significantly increased extracellular 5-HT levels in the NAC and mPFC, respectively, whereas amperozide (1 and 10 mg/kg) increased extracellular 5-HT levels in both regions. Hertel et al. (1997) reported similar results with risperidone and suggested that this might be relevant to its ability to improve negative symptoms. The enhancement of 5-HT efflux in the PFC may contribute to the ability of these agents to improve mood disorders and cognition (Ichikawa et al., 1998). Bortolozzi et al. (2003) reported that clozapine blocked the efflux of cortical 5-HT produced by the hallucinogen DOI in rat brain by both 5-HT_{2A}- and α_1 adrenoceptor dependent mechanisms.

Conclusions

There is now abundant evidence that multiple types of 5-HT_{2A} receptors are of great relevance to the action of the APDs which have supplanted chlorpromazine and other typical APDs as the major treatment for schizophrenia and other types of psychosis because of their superior tolerability and efficacy, in some but not all domains of these complex, heterogeneous syndromes. At least three of the 5-HT receptors appear to act by modulating DA efflux in the cortex, hippocampus, NAC, and most likely, the VTA and SNc as well. Actions at the 5-HT_{2A} receptor, in particular, but also the 5-HT_{1A} and 5-HT_{2C} receptors appear most important in this regard. The 5-HT_{2A} receptor antagonism requires some degree of blockade of D₂ receptor for their action in patients with schizophrenia in an acute psychosis but might be more effective on its own, when achieved with selective 5-HT_{2A} antagonists such as pimavanserin or M100907 to prevent recurrence of psychosis, i.e. as maintenance treatment. The 5-HT_{2A} and 5-HT_{1A} actions of the atypical APDs are mediated through actions on DA, 5-HT, glutamate and GABA neurons; other types of neurons are even glia may also be involved. 5-HT_{2C} agonism and antagonism, as well as 5-HT₆ antagonism, may be relevant features of some atypical APDs or stand alone agents to achieve antipsychotic efficacy and cognitive improvement.

Abbreviations

ACh	acetylcholine
APDs	antipsychotic drugs
BDNF	brain-derived neurotrophic factor
DA	dopamine
EPS	extrapyramidal symptoms
ERK	extracellular-regulated kinase
LI	latent inhibition
LSD	lysergic acid diethylamide
MAP	mitogen-activated protein kinase
mPFC	medial prefrontal cortex
NAC	nucleus accumbens
PCP	phencyclidine

PPI	prepulse inhibition
R-SAT	Receptor Selection and Amplification Technology
5-HT	serotonin
SNc	substantia nigra pars compacta
VTA	ventrotergmental area

Acknowledgements

This work was supported by grants from the Ritter Foundation, the Prentiss Foundation and the Hintz Family Foundation.

References

- Abdul-Monim, Z., Reynolds, G.P. and Neill, J.C. (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. *Behav. Brain Res.*, 169(2): 263–273.
- Ahlenius, S. (1989) Antipsychotic-like properties of the 5-HT_{1A} agonist 8-OH-DPAT in the rat. *Pharmacol. Toxicol.*, 64(1): 3–5.
- Anil Yagcioglu, A.E., Kivircik Akdede, B.B., Turgut, T.I., Tumuklu, M., Yazici, M.K., Alptekin, K., Ertugrul, A., Jayathilake, K., Gogus, A., Tunca, Z. and Meltzer, H.Y. (2005) A double-blind controlled study of adjunctive treatment with risperidone in schizophrenic patients partially responsive to clozapine: efficacy and safety. *J. Clin. Psychiatry*, 66: 63–72.
- Arnt, J. and Skarsfeldt, T. (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology*, 18: 63–101.
- Arora, R.C. and Meltzer, H.Y. (1991) Serotonin₂ (5-HT₂) receptor binding in the frontal cortex of schizophrenic patients. *J. Neural. Transm. Gen. Sect.*, 85: 19–29.
- Arranz, M., Collier, D., Sodhi, M., Ball, D., Roberts, G., Price, J., Sham, P. and Kerwin, R. (1995) Association between clozapine response and allelic variation in 5-HT_{2A} receptor gene. *Lancet*, 346(8970): 281–282.
- Arranz, M.J., Munro, J., Owen, M.J., Spurlock, G., Sham, P.C., Zhao, J., Kirov, G., Collier, D.A. and Kerwin, R.W. (1998a) Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT_{2A} receptor gene and response to clozapine. *Mol. Psychiatry*, 3(1): 61–66.
- Arranz, M.J., Munro, J., Sham, P., Kirov, G., Murray, R.M., Collier, D.A. and Kerwin, R.W. (1998b) Meta-analysis of studies on genetic variation in 5-HT_{2A} receptors and clozapine response. *Schizophr. Res.*, 32(2): 93–99.
- Bai, O., Chlan-Fourney, J., Bowen, R., Keegan, D. and Li, X.M. (2003) Expression of brain-derived neurotrophic factor mRNA in rat hippocampus after treatment with antipsychotic drugs. *J. Neurosci. Res.*, 71: 127–131.

- Bakshi, V.P. and Geyer, M.A. (1997) Phencyclidine-induced deficits in prepulse inhibition of startle are blocked by prazosin, an α -1 noradrenergic antagonist. *J. Pharmacol. Exp. Ther.*, 283: 666–674.
- Barbato, L., Monge, A., Stocchi, F. and Nordera, G. (1996) Melperone in the treatment of iatrogenic psychosis in Parkinson's disease. *Funct. Neurol.*, 11(4): 201–207.
- Barker, E.L., Westphal, R.S., Schmidt, D. and Sanders-Bush, E. (1994) Constitutively active 5-hydroxytryptamine_{2C} receptors reveal novel inverse agonist activity of receptor ligands. *J. Biol. Chem.*, 269: 11687–11690.
- Boess, F.G., Monsma, F.J., Jr. and Sleight, A.J. (1998) Identification of residues in transmembrane regions III and VI that contribute to the ligand binding site of the serotonin 5-HT₆ receptor. *J. Neurochem.*, 71(5): 2169–2177.
- Bonaccorso, S., Meltzer, H.Y., Li, Z., Dai, J., Alboszta, A.R. and Ichikawa, J. (2002) SR46349-B, a 5-HT (2A/2C) receptor antagonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Neuropsychopharmacology*, 27: 430–441.
- Bortolozzi, A., Amargos-Bosch, M., Adell, A., Diaz-Mataix, L., Serrats, J., Pons, S. and Artigas, F. (2003) In vivo modulation of 5-hydroxytryptamine release in mouse prefrontal cortex by local 5-HT (2A) receptors: effect of antipsychotic drugs. *Eur. J. Neurosci.*, 18: 1235–1246.
- Bortolozzi, A., Diaz-Mataix, L., Scorza, M.C., Celada, P. and Artigas, F. (2005) The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. *J. Neurochem.*, 95: 1597–1607.
- Bruins Slot, L.A., Kleven, M.S. and Newman-Tancredi, A. (2005) Effects of novel antipsychotics with mixed D(2) antagonist/5-HT(1A) agonist properties on PCP-induced social interaction deficits in the rat. *Neuropharmacology*, 49(7): 996–1006.
- Burnet, P.W., Eastwood, S.L. and Harrison, P.J. (1996) 5-HT_{1A} and 5-HT_{2A} receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology*, 15: 442–455.
- Burris, K.D., Molski, T.F., Xu, C., Ryan, E., Tottori, K., Kikuchi, T., Yocca, F.D. and Molinoff, P.B. (2002) Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D₂ receptors. *J. Pharmacol. Exp. Ther.*, 302(1): 381–389.
- Burstein, E.S., Ma, J., Wong, S., Gao, Y., Pham, E., Knapp, A.E., Nash, N.R., Olsson, R., Davis, R.E., Hacksell, U., Weiner, D.M. and Brann, M.R. (2005) Intrinsic efficacy of antipsychotics at human D₂, D₃, and D₄ dopamine receptors: identification of the clozapine metabolite N-desmethylozapine as a D₂/D₃ partial agonist. *J. Pharmacol. Exp. Ther.*, 315: 1278–1287.
- Carlsson, M.L., Martin, P., Nilsson, M., Sorensen, S.M., Carlsson, A., Waters, S. and Waters, N. (1999) The 5-HT_{2A} receptor antagonist M100907 is more effective in counteracting NMDA antagonist- than dopamine agonist-induced hyperactivity in mice. *J. Neural. Transm.*, 106: 123–129.
- Chung, Y.C., Li, Z., Dai, J., Meltzer, H.Y. and Ichikawa, J. (2004) Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 1023: 54–63.
- Coyle, J.T. (2006) Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol. Neurobiol.*, 26(4–6): 365–384.
- Darmani, N.A., Martin, B.R., Pandey, U. and Glennon, R.A. (1990) Do functional relationships exist between 5-HT_{1A} and 5-HT₂ receptors? *Pharmacol. Biochem. Behav.*, 36: 901–906.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2001) The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. *Neuropsychopharmacology*, 25: 662–668.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2003) Potentiation of amphetamine-induced changes in dopamine and 5-HT by a 5-HT(6) receptor antagonist. *Brain Res. Bull.*, 59: 513–521.
- Dean, B., Hussain, T., Hayes, W., Scarr, E., Kitsoulis, S., Hill, C., Opeskin, K. and Copolov, D.L. (1999) Changes in serotonin_{2A} and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. *J. Neurochem.*, 72(4): 1593–1599.
- De Deurwaerdere, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24(13): 3235–3241.
- De Deurwaerdere, P. and Spampinato, U. (1999) Role of serotonin(2A) and serotonin(2B/2C) receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73(3): 1033–1042.
- Diaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25(47): 10831–10843.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38(8): 1195–1205.
- Doherty, M.D. and Pickel, V.M. (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.*, 864(2): 176–185.
- Dunlop, J., Marquis, K.L., Lim, H.K., Leung, L., Kao, J., Cheesman, C. and Rosenzweig-Lipson, S. (2006) Pharmacological profile of the 5-HT(2C) receptor agonist WAY-163909; therapeutic potential in multiple indications. *CNS Drug Rev.*, 12(3–4): 167–177.
- Farde, L., Nordstrom, A.L., Wiesel, F.A., Pauli, S., Halldin, C. and Sedvall, G. (1992) Positron emission tomographic analysis of central D₁ and D₂ dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch. Gen. Psychiatry*, 49: 538–544.
- Fiorella, D., Helsley, S., Rabin, R.A. and Winter, J.C. (1995) The interactions of typical and atypical antipsychotics with

- the (–)-2,5-dimethoxy-4-methamphetamine (DOM) discriminative stimulus. *Neuropharmacology*, 34: 1297–1303.
- Fischman, A.J., Bonab, A.A., Babich, J.W., Alpert, N.M., Rauch, S.L., Elmaleh, D.R., Shoup, T.M., Williams, S.A. and Rubin, R.H. (1996) Positron emission tomographic analysis of central 5-hydroxytryptamine₂ receptor occupancy in healthy volunteers treated with the novel antipsychotic agent, ziprasidone. *J. Pharmacol. Exp. Ther.*, 279(2): 939–947.
- Frantz, K.J., Hansson, K.J., Stouffer, D.G. and Parsons, L.H. (2002) 5-HT₆ receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. *Neuropharmacology*, 42: 170–180.
- Frederick, J.A. and Meador-Woodruff, J.H. (1999) Effects of clozapine and haloperidol on 5-HT₆ receptor mRNA levels in rat brain. *Schizophr. Res.*, 38: 7–12.
- Gardell, L.R., Vanover, K.E., Pounds, L., Johnson, R.W., Barido, R., Anderson, G.T., Veinbergs, I., Dyssegaard, A., Brunmark, P., Tabatabaei, A., Davis, R.E., Brann, M.R., Hacksell, U. and Bonhaus, D.W. (2007) ACP-103, a 5-hydroxytryptamine 2A receptor inverse agonist, improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. *J. Pharmacol. Exp. Ther.*, 322(2): 862–870.
- Gerard, C., el Mestikawy, S., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23: 164–173.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 746: 207–219.
- Gewirtz, J.C. and Marek, G.J. (2000) Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. *Neuropsychopharmacology*, 23(5): 569–576.
- Geyer, M.A., Krebs-Thomson, K., Braff, D.L. and Swerdlow, N.R. (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl.)*, 156(2–3): 117–154.
- Gleason, S.D. and Shannon, H.E. (1997) Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. *Psychopharmacology*, 129: 79–84.
- Grayson, B., Idris, N.F. and Neill, J.C. (2007) Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behav. Brain Res.*, 184(1): 31–38.
- Gresch, P.J., Strickland, L.V. and Sanders-Bush, E. (2002) Lysergic acid diethylamide-induced Fos expression in rat brain: role of serotonin-2A receptors. *Neuroscience*, 114(3): 707–713.
- Grunder, G., Landvogt, C., Vernaleken, Buchholz, H.-G., Ondracek, J., Siessmeier, Hartter, S., Schreckenberger, M., Stoeter, P., Hiemke, C., Rosch, F., Wong, D.F. and Bartenstein, P. (2006) The striatal and extrastriatal D_{2/3} receptor-binding profile of clozapine in patients with schizophrenia. *Neuropsychopharmacology*, 31(5): 1027–1035.
- Gudelsky, G.A., Koenig, J.I., Simonovic, M., Koyama, T., Ohmori, T. and Meltzer, H.Y. (1987) Differential effects of haloperidol, clozapine and fluperlapine on tuberoinfundibular dopamine neurons and prolactin secretion in the rat. *J. Neural. Transm.*, 68: 227–240.
- Hagger, C., Buckley, P., Kenny, J.T., Friedman, L., Ubogy, D. and Meltzer, H.Y. (1993) Improvement in cognitive functions and psychiatric symptoms in treatment-refractory schizophrenic patients receiving clozapine. *Biol. Psychiatry*, 34: 702–712.
- Hagiwara, H., Fujita, Y., Ishima, T., Kunitachi, S., Shirayama, Y., Iyo, M. and Hashimoto, K. (2007) Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the antipsychotic drug perospirone: role of serotonin 5-HT_{1A} receptors. *Eur. Neuropsychopharmacol.*, E pub ahead of print.
- Hamon, M., Doucet, E., Lefevre, K., Miquel, M.C., Lanfumey, L., Insausti, R., Frechilla, D., Del Rio, J. and Verge, D. (1999) Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT₆ receptors. *Neuropsychopharmacology*, 21: 68S–76S.
- Hasler, F., Grimberg, U., Benz, M.A., Huber, T. and Vollenweider, F.X. (2004) Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. *Psychopharmacology*, 172(2): 145–156.
- Herrick-Davis, K., Grinde, E. and Teitler, M. (2000) Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295(1): 226–232.
- Hertel, P., Nomikos, G.G., Schilström, B., Arborelius, L. and Svensson, T.H. (1997) Risperidone dose-dependently increases extracellular concentrations of serotonin in the rat frontal cortex: role of α_2 adrenoceptor antagonism. *Neuropsychopharmacology*, 17: 44–55.
- Hietala, J., Kuonnamaki, M., Palvimaki, E.P., Laakso, A., Majasuo, H. and Syvalahti, E. (2001) Sertindole is a serotonin 5-HT_{2C} inverse agonist and decreases agonist but not antagonist binding to 5-HT_{2C} receptors after chronic treatment. *Psychopharmacol.*, 157(2): 180–187.
- Honer, W.G., Thornton, A.E., Chen, E.Y., Chan, R.C., Wong, J.O., Bergmann, A., Falkai, P., Pomarol-Clotet, E., McKenna, P.J., Stip, E., Williams, R., MacEwan, G.W., Wasan, K. and Procyshyn, R. (2006) Clozapine and Risperidone Enhancement (CARE) Study Group Clozapine alone versus clozapine and risperidone with refractory schizophrenia. *N. Engl. J. Med.*, 354: 472–482.
- Hong, C.J., Yu, Y.W., Lin, C.H. and Tsai, S.J. (2003) An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. *Neurosci. Lett.*, 349: 206–208.
- Huang, M., Li, Z., Prus, A.J., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2005) 5-HT_{2A} and 5-HT_{2C} receptor antagonism

- enhances risperidone-induced dopamine (DA) efflux in rat medial prefrontal cortex (mpfc) and diminishes it in the nucleus accumbens (NAC). *Neurosci. Abs.* 914.10.
- Huber, G., Maerz, W., Martin, J.R., Malherbe, P., Richards, J.G., Sueoka, N., Ohm, T. and Hoffmann, M.M. (2000) Characterization of transgenic mice expressing apolipoprotein E4 (C112R) and apolipoprotein E4 (L28P; C112R). *Neuroscience*, 101: 211–218.
- Hurlemann, R., Matusch, A., Kuhn, K.U., Berning, J., Elmenhorst, D., Winz, O., Kolsch, H., Zilles, K., Wagner, M., Maier, W. and Bauer, A. (2008) 5-HT_{2A} receptor density is decreased in the at-risk mental state. *Psychopharmacology (Berl.)*, 195(4): 579–590.
- Ichikawa, J., Dai, J., O'Laughlin, I.A., Dai, J., Fowler, W. and Meltzer, H.Y. (2002a) Atypical, but not typical, antipsychotic drugs selectively increase acetylcholine release in rat medial prefrontal cortex, nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 325–339.
- Ichikawa, J., Ishii, H., Bonaccorso, S., Fowler, W.L., O'Laughlin, I.A. and Meltzer, H.Y. (2001) 5-HT_{2A} and D(2) receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.*, 76(5): 1521–1531.
- Ichikawa, J., Kuroki, T., Dai, J. and Meltzer, H.Y. (1998) Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. *Eur. J. Pharmacol.*, 351: 163–171.
- Ichikawa, J., Li, Z., Dai, J. and Meltzer, H.Y. (2002b) Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 956(2): 349–357.
- Ichikawa, J. and Meltzer, H.Y. (1995) DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. *Brain Res.*, 698: 204–208.
- Ichikawa, J. and Meltzer, H.Y. (2000) The effect of serotonin(1A) receptor agonism on antipsychotic drug-induced dopamine release in rat striatum and nucleus accumbens. *Brain Res.*, 858(2): 252–263.
- Ishikane, T., Kusumi, I., Matsubara, R., Matsubara, S. and Koyama, T. (1997) Effects of serotonergic agents on the up-regulation of dopamine D₂ receptors induced by haloperidol in rat striatum. *Eur. J. Pharmacol.*, 321: 163–169.
- Jakab, R.L. and Goldman-Rakic, P.S. (1998) 5-Hydroxytryptamine_{2A} serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc. Natl. Acad. Sci. USA*, 95: 735–740.
- Joyce, J.N., Shane, A., Lexow, N., Winokur, A., Casanova, M.F. and Kleinman, J.E. (1993) Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. *Neuropsychopharmacology*, 8: 315–336.
- Kapur, S., Remington, G., Zipursky, R.B., Wilson, A.A. and Houle, S. (1995) The D₂ dopamine receptor occupancy of risperidone and its relationship to extrapyramidal symptoms: a PET study. *Life Sci.*, 57: PL103–PL107.
- Kapur, S. and Seeman, P. (2000) Antipsychotic agents differ in how fast they come off the dopamine D₂ receptors. Implications for atypical antipsychotic action. *J. Psychiatry Neurosci.*, 25: 161–166.
- Kapur, S., Zipursky, R., Jones, C., Shammi, C.S., Remington, G. and Seeman, P. (2000) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D₂ receptor occupancy. *Arch. Gen. Psychiatry*, 57: 553–559.
- Kapur, S., Zipursky, R.B., Remington, G., Jones, C., DaSilva, J., Wilson, A.A. and Houle, S. (2005) 5-HT₂ and D₂ receptor occupancy of olanzapine in schizophrenia: a PET investigation. *Schizophr. Res.*, 76(2–3): 357–358.
- Kessler, R.M., Ansari, M.S., Riccardi, P., Li, R., Jayatilake, K., Dawant, B. and Meltzer, H.Y. (2005) Occupancy of striatal and extrastriatal dopamine D(2)/D(3) receptors by olanzapine and haloperidol. *Neuropsychopharmacology*, 30(12): 2283–2289.
- Kessler, R.M., Ansari, M.S., Riccardi, P., Li, R., Jayatilake, K., Dawant, B. and Meltzer, H.Y. (2006) Occupancy of striatal and extrastriatal dopamine D₂ receptors by clozapine and quetiapine. *Neuropsychopharmacology*, 31: 1991–2001.
- Kohen, R., Metcalf, M.A., Khan, N., Druck, T., Huebner, K., Lachowicz, J.E., Meltzer, H.Y., Sibley, D.R., Roth, B.L. and Hamblin, M.W. (1996) Cloning, characterization, and chromosomal localization of a human 5-HT₆ serotonin receptor. *J. Neurochem.*, 66: 47–56.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (1999) Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J. Pharmacol. Exp. Ther.*, 288(2): 774–781.
- Lacroix, L.P., Dawson, L.A., Hagan, J.J. and Heidbreder, C.A. (2004) 5-HT₆ receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. *Synapse*, 51: 158–164.
- Leng, A., Ouagazzal, A., Feldon, J. and Higgins, G.A. (2003) Effect of the 5-HT₆ receptor antagonists Ro04-6790 and Ro65-7199 on latent inhibition and prepulse inhibition in the rat: comparison to clozapine. *Pharmacol. Biochem. Behav.*, 75(2): 281–288.
- Li, Z., Huang, M., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2005a) N-desmethyloclazapine, a major metabolite of clozapine, increases cortical acetylcholine and dopamine release in vivo via stimulation of M1 muscarinic receptors. *Neuropsychopharmacology*, 30(11): 1986–1995.
- Li, Z., Huang, M., Prus, A., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2005b) Effect of the 5-HT_{2C} receptor antagonist SB242084 in combination with haloperidol and the 5-HT_{2A/2C} inverse agonist ACP103 on dopamine (DA) efflux in rat brain. *Neurosci. Abs.*, 914.8.
- Li, Z., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2004) Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. *Eur. J. Pharmacol.*, 493: 75–83.
- Lieben, C.K., Blokland, A., Sik, A., Sung, E., van Nieuwenhuizen, P. and Schreiber, R. (2005) The selective 5-HT₆

- receptor antagonist Ro4368554 restores memory performance in cholinergic and serotonergic models of memory deficiency in the rat. *Neuropsychopharmacology*, 30(12): 2169–2179.
- Liegeois, J-F., Ichikawa, J. and Meltzer, H.Y. (2002) 5HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res.*, 947: 157–165.
- Linden, A.M., Vaisanen, J., Lakso, M., Nawa, H., Wong, G. and Castren, E. (2000) Expression of neurotrophins BDNF and NT-3, and their receptors in rat brain after administration of antipsychotic and psychotropic agents. *J. Mol. Neurosci.*, 14: 27–37.
- Mamo, D., Kapur, S., Keshavan, M., Laruelle, M., Taylor, C.C., Kothare, P.A., Barsoum, P. and McDonnell, D. (2008a) D₂ receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. *Neuropsychopharmacology*, 33(2): 298–304.
- Mamo, D., Kapur, S., Shammi, C.M., Papatheodorou, G., Mann, S., Therrien, F. and Remington, G. (2004) A PET study of dopamine D₂ and serotonin 5-HT₂ receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone. *Am. J. Psychiatry*, 161: 818–825.
- Mamo, D.C., Uchida, H., Vitcu, I., Barsoum, P., Gendron, A., Goldstein, J. and Kapur, S. (2008b) Quetiapine extended-release versus immediate-release formulation: a positron emission tomography study. *J. Clin. Psychiatry*, 69(1): 81–86.
- Marder, S.R. (1994) Risperidone in the treatment of schizophrenia. *Am. J. Psychiatry*, 151: 825–835.
- Martin, P., Waters, N., Waters, S., Carlsson, A. and Carlsson, M.L. (1997) MK-801-induced hyperlocomotion: differential effects of M 100907, SDZ PSD 958 and raclopride. *Eur. J. Pharmacol.*, 335(2–3): 107–116.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Nguyen, H.Q., Dawson, L.A., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 ((7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole) – a novel 5-HT_{2C} receptor selective agonist with preclinical antipsychotic activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.
- Masellis, M., Basile, V., Meltzer, H.Y., Lieberman, J.A., Sevy, S., Macciardi, F.M., Cola, P., Howard, A., Badri, F., Nothen, M.M., Kalow, W. and Kennedy, J.L. (1998) Serotonin subtype 2 receptor genes and clinical response to clozapine in schizophrenia patients. *Neuropsychopharmacology*, 19: 123–132.
- Matsubara, S. and Meltzer, H.Y. (1989) Effect of typical and atypical antipsychotic drugs on 5-HT₂ receptor density in rat cerebral cortex. *Life Sci.*, 45(15): 1397–1406.
- Matsumoto, M., Togashi, H., Mori, K., Ueno, K., Miyamoto, A. and Yoshioka, M. (1999) Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. *Eur. J. Pharmacol.*, 383: 39–48.
- Meltzer, H.Y. (1995) Role of serotonin in the action of atypical antipsychotic drugs. *Clin. Neurosci.*, 3(2): 4–75.
- Meltzer, H.Y. (2002) Mechanism of action of atypical antipsychotic drugs. In: Davis K.L., Charney D., Coyle J.T. and Nemeroff C. (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress*. Lippincott Williams & Wilkins, Philadelphia, pp. 819–832.
- Meltzer, H.Y., Arvanitis, L., Bauer, D., Rein, W. and Meta-Trial Study Group. (2004) Placebo-controlled evaluation of four novel compounds for the treatment of schizophrenia and schizoaffective disorder. *Am. J. Psychiatry*, 161(6): 975–984.
- Meltzer, H.Y. and Fatemi, S.H. (1996) The role of serotonin in schizophrenia and the mechanism of action on anti-psychotic drugs. In: Kane J.M., Moller H.J. and Awouters F. (Eds.), *Serotonergic Mechanisms in Antipsychotic Treatment*. Marcel Dekker, New York, pp. 77–107.
- Meltzer, H.Y. and Maes, M. (1995) Pindolol pretreatment blocks stimulation by meta-chlorophenylpiperazine of prolactin but not cortisol secretion in normal men. *Psychiatry Res.*, 58: 89–98.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251: 238–246.
- Meltzer, H.Y. and Nash, J.F. (1991) Effects of antipsychotic drugs on serotonin receptors. *Pharmacol. Rev.*, 43: 587–604.
- Meneses, A. (2001) Effects of the 5-HT₆ receptor antagonist Ro 04-6790 on learning consolidation. *Behav. Brain Res.*, 118: 107–110.
- Meneses, A. (2002) Involvement of 5-HT_{2A/2B/2C} receptors on memory formation: simple agonism, antagonism, or inverse agonism? *Cell. Mol. Neurobiol.*, 22(5–6): 675–688.
- Millan, M.J., Gobert, A., Newman-Tancredi, A., Audinot, V., Lejeune, F., Rivet, J.M., Cussac, D., Nicolas, J.P., Muller, O. and Lavielle, G. (1998) S 16924 ((R)-2-[1-[2-(2,3-dihydrobenzo[1,4] dioxin-5-Yloxy)-ethyl]-pyrrolidin-3-yl]-1-(4-fluorophenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)_{1A} agonist properties: I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol. *J. Pharmacol. Exp. Ther.*, 286: 1341–1355.
- Millan, M.J., Gobert, A., Roux, S., Porsolt, R., Meneses, A., Carli, M., Di Cara, B., Jaffard, R., Rivet, J.M., Lestage, P., Mocaer, E., Peglion, J.L. and Dekeyne, A. (2004) The serotonin_{1A} receptor partial agonist S15535 [4-(benzodioxan-5-yl)-1-(indan-2-yl)piperazine] enhances cholinergic transmission and cognitive function in rodents: a combined neurochemical and behavioral analysis. *J. Pharmacol. Exp. Ther.*, 311: 190–203.
- Minabe, Y., Shirayama, Y., Hashimoto, K., Routledge, C., Hagan, J.J. and Ashby, C.R., Jr. (2004) Effect of the acute and chronic administration of the selective 5-HT₆ receptor antagonist SB-271046 on the activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. *Synapse*, 52(1): 20–28.
- Miyamoto, S., Duncan, G.E., Marx, C.E. and Lieberman, J.A. (2005) Treatments for schizophrenia: a critical review of

- pharmacology and mechanisms of action of antipsychotic drugs. *Mol. Psychiatry*, 10(1): 79–104.
- Moghaddam, B. and Bunney, B.S. (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. *J. Neurochem.*, 54(5): 1755–1760.
- Monsma, F.J., Jr., Shen, Y., Ward, R.P., Hamblin, M.W. and Sibley, D.R. (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.*, 43: 320–327.
- Newman-Tancredi, A., Cussac, D. and Depoortere, R. (2007) Neuropharmacological profile of bifeprunox: merits and limitations in comparison with other third-generation antipsychotics. *Curr. Opin. Investig. Drugs*, 8(7): 539–554.
- Newman-Tancredi, A., Gavaudan, S., Conte, C., Chaput, C., Touzard, M., Verrielle, L., Audinot, V. and Millan, M.J. (1998) Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [³⁵S]GTPgammaS binding study. *Eur. J. Pharmacol.*, 355: 245–256.
- Ngan, E.T., Yatham, L.N., Ruth, T.J. and Liddle, P.F. (2000) Decreased serotonin 2A receptor densities in neuroleptic-naïve patients with schizophrenia: a PET study using [(18)F]setoperone. *Am. J. Psychiatry*, 157: 1016–1018.
- Ninan, I. and Kulkarni, S.K. (1998) 5-HT_{2A} receptor antagonists block MK-801-induced stereotypy and hyperlocomotion. *Eur. J. Pharmacol.*, 358: 111–116.
- Niswender, C.M., Copeland, S.C., Herrick-Davis, K., Emeson, R.B. and Sanders-Bush, E. (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, 274(14): 9472–9478.
- Olijslagers, J.E., Perlstein, B., Werkman, T.R., McCreary, A.C., Siarey, R., Kruse, C.G. and Wadman, W.J. (2005) The role of 5-HT(2A) receptor antagonism in amphetamine-induced inhibition of A10 dopamine neurons in vitro. *Eur. J. Pharmacol.*, 520(1–3): 77–85.
- Okuyama, S., Chaki, S., Kawashima, N., Suzuki, Y., Ogawa, S., Kumagai, T., Nakazato, A., Nagamine, M., Yamaguchi, K. and Tomisawa, K. (1997a) The atypical antipsychotic profile of NRA0045, a novel dopamine D₄ and 5-hydroxytryptamine_{2A} receptor antagonist, in rats. *Brit. J. Pharmacol.*, 121(3): 515–525.
- Okuyama, S., Chaki, S., Yoshikawa, R., Suzuki, Y., Ogawa, S., Imagawa, Y., Kawashima, N., Ikeda, Y., Kumagai, T., Nakazato, A., Nagamine, M. and Tomisawa, K. (1997b) In vitro and in vivo characterization of the dopamine D₄ receptor, serotonin 5-HT_{2A} receptor and alpha-1 adrenoceptor antagonist (R)-(+)-2-amino-4-(4-fluorophenyl)-5-[1-[4-(4-fluorophenyl)-4-oxobutyl]pyrrolidin-3-yl]thiazole (NRA0045). *J. Pharmacol. Exp. Ther.*, 282(1): 56–63.
- Parada, M.A., Hernandez, L., Puig de Parada, M., Rada, P. and Murzi, E. (1997) Selective action of acute systemic clozapine on acetylcholine release in the rat prefrontal cortex by reference to the nucleus accumbens and striatum. *J. Pharmacol. Exp. Ther.*, 281: 582–588.
- Pazos, A., Probst, A. and Palacios, J.M. (1987) Serotonin receptors in the human brain – IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*, 21: 123–139.
- Pehek, E.A., McFarlane, H.G., Maguschak, K., Price, B. and Pluto, C.P. (2001) M100907, a selective 5-HT(2A) antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res.*, 888(1): 51–59.
- Pouzet, B., Didriksen, M. and Arnt, J. (2002) Effects of the 5-HT(6) receptor antagonist, SB-271046, in animal models for schizophrenia. *Pharmacol. Biochem. Behav.*, 71(4): 635–643.
- Prinssen, E.P., Ellenbroek, B.A. and Cools, A.R. (1994) Combined antagonism of adrenoceptors and dopamine and 5-HT receptors underlies the atypical profile of clozapine. *Eur. J. Pharmacol.*, 262: 167–170.
- Prinssen, E.P., Kleven, M.S. and Koek, W. (1996) Effects of dopamine antagonists in a two-way active avoidance procedure in rats: interactions with 8-OH-DPAT, ritanserin, and prazosin. *Psychopharmacology*, 128: 191–197.
- Reynolds, G.P., Templeman, L.A. and Zhang, Z.J. (2005) The role of 5-HT_{2C} receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 29(6): 1021–1028.
- Richtand, N.M., Welge, J.A., Logue, A.D., Keck, P.E., Jr., Strakowski, S.M. and McNamara, R.K. (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. *Neuropsychopharmacology*, 32(8): 1715–1726.
- Riemer, C., Borroni, E., Levet-Trafit, B., Martin, J.R., Poli, S., Porter, R.H. and Bos, M. (2003) Influence of the 5-HT₆ receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT₆ receptor antagonist. *J. Med. Chem.*, 46(7): 1273–1276.
- Rinaldi-Carmona, M., Congy, C., Simiand, J., Oury-Donat, F., Soubrie, P., Breliere, J.C. and Le Fur, G. (1993) Repeated administration of SR 46349B, a selective 5-hydroxytryptamine₂ antagonist, up-regulates 5-hydroxytryptamine₂ receptors in mouse brain. *Mol. Pharmacol.*, 3(1): 84–89.
- Rogers, D.C. and Hagan, J.J. (2001) 5-HT₆ receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology*, 158: 114–119.
- Rogers, D.C., Robinson, T.L., Quilter, A.J., Routledge, C. and Hagan, J.J. (1999) Cognitive enhancement effects of the selective 5-HT₆ antagonist SB-271046. *Br. J. Pharmacol.*, 127: p. 22.
- Rollema, H., Lu, Y., Schmidt, A.W. and Zorn, S.H. (1997) Clozapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. *Eur. J. Pharmacol.*, 338: R3–R5.
- Roth, B.L., Ciaranello, R.D. and Meltzer, H.Y. (1992) Binding of typical and atypical antipsychotic agents to transiently expressed 5-HT_{1C} receptors. *J. Pharmacol. Exp. Ther.*, 260: 1361–1365.
- Roth, B.L., Craig, S.C., Choudhary, M.S., Uluer, A., Monsma, F.J., Jr., Shen, Y., Meltzer, H.Y. and Sibley, D.R. (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.*, 268: 1403–1410.

- Ruat, M., Traiffort, E., Arrang, J.M., Tardivel-Lacombe, J., Diaz, J., Leurs, R. and Schwartz, J.C. (1993) A novel rat serotonin (5-HT₆) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.*, 193: 268–276.
- Santana, N., Bortolozzi, A., Serrats, J., Mengod, G. and Artigas, F. (2004) Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex*, 14(10): 1100–1109.
- Schmidt, C.J., Sorensen, S.M., Kehne, J.H., Carr, A.A. and Palfreyman, M.G. (1995) The role of 5-HT_{2A} receptors in antipsychotic activity. *Life Sci.*, 56: 2209–2222.
- Schotte, A., Janssen, P.F., Gommeren, W., Luyten, W.H., Van Gompel, P., Lesage, A.S., De Loore, K. and Leysen, J.E. (1996) Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology*, 124: 57–73.
- Shipley, J. (1998) M100907 phase IIB trial Hoechst Marion Roussel Conference on M100907, West Palm Beach, Florida.
- Shirazi-Southall, S., Rodriguez, D.E. and Nomikos, G.G. (2002) Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. *Neuropsychopharmacology*, 26(5): 583–594.
- Sleight, A.J., Consolo, S., Martin, J.R., Boess, J.C., Bentley, J.C. and Bourson, A. (1999) 5-HT₆ receptors: functional correlates and potential therapeutic indications. *Behav. Pharmacol.*, 10: S86–S87.
- Sodhi, M.S., Airey, D.C., Lambert, W., Burnet, P.W., Harrison, P.J. and Sanders-Bush, E. (2005) A rapid new assay to detect RNA editing reveals antipsychotic-induced changes in serotonin-2C transcripts. *Mol. Pharmacol.*, 68(3): 711–719.
- Sodhi, M.S., Arranz, M.J., Curtis, D., Ball, D.M., Sham, P., Roberts, G.W., Price, J., Collier, D.A. and Kerwin, R.W. (1995) Association between clozapine response and allelic variation in the 5-HT_{2C} receptor gene. *Neuroreport*, 7(1): 169–172.
- Stahl, S.M. and Grady, M.M. (2004) A critical review of atypical antipsychotic utilization: comparing monotherapy with polypharmacy and augmentation. *Curr. Med. Chem.*, 11(3): 313–327.
- Stean, T.O., Hirst, W.D., Thomas, D.R., Price, G.W., Rogers, D., Riley, G., Bromidge, S.M., Serafinowska, H.T., Smith, D.R., Bartlett, S., Deeks, N., Duxon, M. and Upton, N. (2002) Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT(6) receptor antagonist. *Pharmacol. Biochem. Behav.*, 71: 645–654.
- Swainston Harrison, T. and Perry, C.M. (2004) Aripiprazole: a review of its use in schizophrenia and schizoaffective disorder. *Drugs*, 64(15): 1715–1736.
- Talvik, M., Nordstrom, A.L., Nyberg, S., Olsson, H., Halldin, C. and Farde, L. (2001) No support for regional selectivity in clozapine-treated patients: a PET study with [(11)C]raclopride and (11)C]FLB 457. *Am. J. Psychiatry*, 158(6): 926–930.
- Tamminga, C.A. (2002) Partial dopamine agonists in the treatment of psychosis. *J. Neural. Transm.*, 109(3): 411–420.
- Trichard, C., Paillere-Martinot, M.L., Attar-Levy, D., Blin, J., Feline, A. and Martinot, J.L. (1998a) No serotonin 5-HT_{2A} receptor density abnormality in the cortex of schizophrenic patients studied with PET. *Schizophr. Res.*, 31: 13–17.
- Trichard, C., Paillere-Martinot, M.L., Attar-Levy, D., Recassens, C., Monnet, F. and Martinot, J.L. (1998b) Binding of antipsychotic drugs to cortical 5-HT_{2A} receptors: a PET study of chlorpromazine, clozapine, and amisulpride in schizophrenic patients. *Am. J. Psychiatry*, 155(4): 505–508.
- Ukai, W., Ozawa, H., Tateno, M., Hashimoto, E. and Saito, T. (2004) Neurotoxic potential of haloperidol in comparison with risperidone: implication of Akt-mediated signal changes by haloperidol. *J. Neural. Transm.*, 111(6): 667–681.
- Vaidya, V.A., Marek, G.J., Aghajanian, G.K. and Duman, R.S. (1997) 5-HT_{2A} receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J. Neurosci.*, 17: 2785–2795.
- Vanover, K.E., Weiner, D.M., Makhay, M., Veinbergs, I., Gardell, L.R., Lameh, J., Del Tredici, A.L., Piu, F., Schiffer, H.H., Ott, T.R., Burstein, E.S., Uldam, A.K., Thygesen, M.B., Schlienger, N., Andersson, C.M., Son, T.Y., Harvey, S.C., Powell, S.B., Geyer, M.A., Tolf, B.R., Brann, M.R. and Davis, R.E. (2006) Pharmacological and behavioral profile of ACP-103, a novel 5-HT_{2A} receptor inverse agonist. *J. Pharmacol. Exp. Ther.*, 317(2): 910–918.
- Varty, G.B. and Higgins, G.A. (1995) Reversal of dizocilpine-induced disruption of prepulse inhibition of an acoustic startle response by the 5-HT₂ receptor antagonist ketanserin. *Eur. J. Pharmacol.*, 287: 201–205.
- Verhoeff, N.P., Meyer, J.H., Kecojevic, A., Hussey, D., Lewis, R., Tauscher, J., Zipursky, R.B. and Kapur, S. (2000) A voxel-by-voxel analysis of [18F]setoperone PET data shows no substantial serotonin 5-HT(2A) receptor changes in schizophrenia. *Psychiatry Res.*, 99: 123–135.
- Vollenweider, F.X., Csomor, P.A., Knappe, B., Geyer, M.A. and Quednow, B.B. (2007) The effects of the preferential 5-HT_{2A} agonist psilocybin on prepulse inhibition of startle in healthy human volunteers depend on interstimulus interval. *Neuropsychopharmacology*, 32(9): 1876–1887.
- Wadenberg, M.L. (1992) Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. *J. Neural. Transm. Gen. Sect.*, 89: 49–59.
- Wadenberg, M.L. and Ahlenius, S. (1991) Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. *J. Neural. Transm. Gen. Sect.*, 83: 43–53.
- Wadenberg, M.L., Hicks, P.B., Richter, J.T. and Young, K.A. (1998) Enhancement of antipsychotic-like properties of raclopride in rats using the selective serotonin2A receptor antagonist MDL 100907. *Biol. Psychiatry*, 44: 508–515.
- Wadenberg, M.L. and Hillegaart, V. (1995) Stimulation of median, but not dorsal, raphe 5-HT_{1A} autoreceptors by the local application of 8-OH-DPAT reverses raclopride-induced catalepsy in the rat. *Neuropharmacology*, 34: 495–499.

- Weiner, D.M., Burstein, E.S., Nash, N., Croston, G.E., Currier, E.A., Vanover, K.E., Harvey, S.C., Donohue, E., Hansen, H.C., Andersson, C.M., Spalding, T.A., Gibson, D.F.C., Krebs-Thomson, K., Powell, S.B., Geyer, M.A., Hacksell, U. and Brann, M.R. (2001) 5-hydroxytryptamine_{2A} receptor inverse agonists as antipsychotics. *J. Pharmacol. Exp. Ther.*, 299: 268–276.
- Woodward, N.D., Purdon, S.E., Meltzer, H.Y. and Zald, D.H. (2005) A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. *Int. J. Neuropsychopharmacol.*, 8: 457–472.
- Woolley, M.L., Marsden, C.A., Sleight, A.J. and Fone, K.C. (2003) Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT₆ receptor antagonist, Ro 04-6790. *Psychopharmacology*, 170: 358–367.
- Yamada, S., Harano, M., Annoh, N., Nakamura, K. and Tanaka, M. (1999) Involvement of serotonin 2A receptors in phencyclidine-induced disruption of prepulse inhibition of the acoustic startle in rats. *Biol. Psychiatry*, 46: 832–838.
- Youngren, K.D., Inglis, F.M., Pivrotto, P.J., Jedema, H.P., Bradberry, C.W., Goldman-Rakic, P.S., Roth, R.H. and Moghaddam, B. (1999) Clozapine preferentially increases dopamine release in the rhesus monkey prefrontal cortex compared with the caudate nucleus. *Neuropsychopharmacology*, 20(5): 403–412.
- Youngren, K.D., Moghaddam, B., Bunney, B.S. and Roth, R.H. (1994) Preferential activation of dopamine overflow in prefrontal cortex produced by chronic clozapine treatment. *Neurosci. Lett.*, 165(1–2): 41–44.
- Zahorodna, A., Bobula, B., Grzegorzewska, M., Tokarski, K. and Hess, G. (2004) The influence of repeated administration of clozapine and haloperidol on the effects of the activation of 5-HT(1A), 5-HT(2) and 5-HT(4) receptors in rat frontal cortex. *J. Physiol. Pharmacol.*, 55(2): 371–379.
- Zocchi, A., Fabbri, D. and Heidbreder, C.A. (2005) Aripiprazole increases dopamine but not noradrenaline and serotonin levels in the mouse prefrontal cortex. *Neurosci. Lett.*, 387(3): 157–161.

CHAPTER 10

Neuropharmacology of second-generation antipsychotic drugs: a validity of the serotonin–dopamine hypothesis

Toshihide Kuroki*, Naoko Nagao and Tatsuo Nakahara

Clinical Research Division, Hizen Psychiatric Center, 160 Yoshinogari, Kanzaki, Saga 842-0192, Japan

Abstract: Newer atypical antipsychotic drugs such as risperidone, olanzapine, quetiapine, ziprasidone and aripiprazole that have been modelled on the prototype agent clozapine and developed since the 1990s are now referred to as second-generation antipsychotic drugs (SGAs). It has been proposed that the interaction between serotonin (5-HT) and dopamine systems may play a critical role in the mechanism of action of atypical antipsychotic drugs because a relatively potent blockade of 5-HT_{2A} receptors coupled with the weaker antagonism of the dopamine D₂ receptors is found to be the only pharmacological feature which most atypical antipsychotic drugs have in common. This so-called ‘serotonin–dopamine hypothesis’ has become a useful model for developing new SGAs to achieve superior antipsychotic efficacy with a lower incidence of extrapyramidal side effects compared to those with first-generation antipsychotic drugs (FGAs) such as haloperidol and chlorpromazine, although it has not been validated yet. In contrast, it has been proposed as the alternative ‘fast-off’ theory according to which atypical profile of SGAs can be determined by the loose D₂-binding kinetics alone, while the blockade of the 5-HT_{2A} receptor may be neither necessary nor sufficient. This chapter reviews the current issues on the serotonin–dopamine hypothesis together with further advances in research on the role of 5-HT receptor subtypes in the mechanism of action for SGAs. In particular, SGA-induced dopamine release in the prefrontal cortex, possibly through the functional activation of 5-HT_{1A} receptors by 5-HT_{2A} and D₂ receptor-mediated interaction, has been thought to be the basis for the neurocognitive effects of these drugs on schizophrenia. Thus, the novel antipsychotic aripiprazole may not only be a simply partial D₂ agonist but also a significant 5-HT_{1A} agonist and 5-HT_{2A} antagonist. These complex properties of antipsychotic aripiprazole may contribute to dopaminergic activation of the local circuitry in the prefrontal cortex of schizophrenic patients.

Keywords: dopamine D₂ receptor; second-generation antipsychotic; serotonin–dopamine hypothesis; 5-HT_{1A} receptor; 5-HT_{2A} receptor

Introduction

Kane et al. (1988) demonstrated that the atypical antipsychotic clozapine was superior to the typical antipsychotic drugs such as haloperidol and

*Corresponding author. Tel.: +81-952-52-3231; Fax: +81-952-53-2864; E-mail: rinkenbucyou@hizen2.hosp.go.jp

chlorpromazine for improving both positive and negative symptoms of treatment-resistant schizophrenia while producing few incidences of extrapyramidal symptoms (EPS). Thereafter, the general use of clozapine was approved in the United States and many other countries, with the requirement of weekly blood monitoring because of its fatal side effect: agranulocytosis. Newer atypical antipsychotic drugs such as risperidone, olanzapine, quetiapine and ziprasidone that have been modelled on clozapine and developed since the 1990s are now referred to as second-generation antipsychotic drugs (SGAs) (Lohr and Braff, 2003). Today, SGAs, except for the prototype agent clozapine, are chosen for the first-line treatment of schizophrenia.

For the past two decades, neuropharmacological studies on SGAs have generally focused on the mechanism of action by which clozapine as well as SGAs can produce superior therapeutic efficacy. The serotonin–dopamine hypothesis was originally proposed by Meltzer (1989) and since then it has become a representative of the theories regarding the pharmacological basis of SGAs. Meltzer (1989) suggests that the interaction between serotonin (5-HT) and dopamine systems may play an important role in the mechanism of action of atypical antipsychotic drugs because the relatively potent blockade of 5-HT_{2A} receptors coupled with the weaker antagonism of dopamine D₂ receptors has been found to be the only pharmacological feature which most atypical antipsychotic drugs share (Meltzer et al., 1989). This hypothesis has become a useful model for developing new SGAs to achieve superior antipsychotic efficacy with a lower incidence of EPS compared to first-generation antipsychotic drugs (FGAs), formerly called typical antipsychotic drugs. However, since the latter half of the 1990s, some neuroimaging studies on in vivo occupancies of D₂ and 5-HT_{2A} receptors by SGAs in medicated patients have disputed the validity of the serotonin–dopamine hypothesis. Based on these findings, Kapur and Seeman (2001) argue the alternative hypothesis that the difference between typical and atypical antipsychotic drugs may be fully explained by the pharmacokinetics of their interaction with the D₂ receptor alone. This ‘fast-off’ theory has provoked

controversy about the role of 5-HT_{2A} receptors in the mechanism of antipsychotic actions of SGAs.

The purpose of this review is to consider the current issues on the serotonin–dopamine hypothesis and the possible role of 5-HT receptor subtypes in the pharmacological basis for clinical effects of SGAs.

SGAs and the serotonin–dopamine hypothesis

Clozapine, a prototype of SGAs, is exactly ‘atypical’ with regard to the binding potency to D₂ receptors, which is much weaker for clozapine than for typical antipsychotic drugs (Farde and Nordström, 1992; Seeman, 1995; Pilowsky et al., 1997). Based on the initial finding that clozapine rapidly induces down-regulation of 5-HT_{2A} receptors in the rat cerebral cortex (Matsubara and Meltzer, 1989), Meltzer et al. (1989) examined in vitro binding potencies of a number of antipsychotic drugs to D₁, D₂ and 5-HT_{2A} receptors. In a comparison of 20 typical antipsychotic drugs, they determined pK_i values (the negative logarithms of the dissociation constant K_i values) for each receptor of 17 atypical antipsychotic drugs that produce a low incidence of EPS in clinical subjects and/or have no or little ability to induce catalepsy in experimental animals. A discriminant analysis to determine the independent contribution of each pK_i value for a given binding site with reference to the classification as a typical or atypical antipsychotic drug revealed that the major contributors to the discriminant function were relatively higher 5-HT_{2A} pK_i values than D₂ pK_i values. D₁ pK_i values did not contribute to the discriminant function. Although chlorpromazine and spiroperidol had high pK_i values for 5-HT_{2A} receptors, they were not classified as atypical antipsychotic drugs because of comparable or greater pK_i values for D₂ receptors. Thus, atypical antipsychotic drugs may share common features such as relatively greater 5-HT_{2A} receptor binding potency than D₂ receptor binding potency (the difference between 5-HT_{2A} pK_i and D₂ pK_i is approximately over one, that is more than 10 times greater affinity for 5-HT_{2A} than for D₂ receptors), while only one factor, such as high 5-HT_{2A} receptor binding

potency or low D_2 receptor binding potency, may not be a sufficient condition. This pharmacological profile of atypical antipsychotic drugs has been thought to provide strong evidence for the serotonin–dopamine hypothesis that postulates a major contribution of the 5-HT_{2A}/ D_2 receptor interactions to the mechanism of action of these drugs (Meltzer, 1989; Meltzer et al., 1989).

Most SGAs released since the 1990s were chosen for development based on the pharmacological features as proposed by Meltzer et al. (1989) (Table 1). Risperidone, the first emerging drug of SGAs, displays strong binding potency to D_2 receptors at an equal level to haloperidol, while the binding potency of this drug to 5-HT_{2A} receptors is much higher than that to D_2 receptors (Leysen et al., 1993; Schotte et al., 1996). This is consistent with the clinical feature that the incidence of EPS with risperidone is dose-related and comparable to haloperidol at higher doses (more than 10 mg/day) because of the extremely high D_2 potency. However, despite moderate potency of quetiapine to 5-HT_{2A} receptors, this drug is classified as an atypical one because of relatively lower potency to

D_2 than to 5-HT_{2A} receptors. In addition, the novel antipsychotic aripiprazole is known as a partial agonist to D_2 receptors ($K_i=0.74$), while it also has significant affinity for 5-HT_{2A} receptors ($K_i=8.7$) (Shapiro et al., 2003). Taking this into account, the net antagonism of D_2 receptors by aripiprazole may be less potent than that of 5-HT_{2A} receptors.

It should be noted that not all SGAs fit the model of the serotonin–dopamine hypothesis. Exceptions include the selective $D_{2/3}$ receptor antagonists such as the substituted benzamide amisulpride, which has been reported to improve both positive and negative symptoms of schizophrenia while producing a minimal incidence of EPS (Leucht et al., 2002). Potent and selective blockade of D_3 receptors rather than D_2 receptors by the substituted benzamides may contribute to their atypical profile such as lesser propensity to produce EPS (Sokoloff et al., 1990).

As discussed, most, but not all, SGAs widely available in the clinical setting at present are atypical antipsychotic drugs with relatively higher affinity for 5-HT_{2A} receptors than for D_2 receptors. A number of compounds that fit the same pharmacological model have also proven to have an atypical antipsychotic profile in clinical trials (Meltzer et al., 2003). To date, these facts have strongly endorsed the validity of the serotonin–dopamine hypothesis. However, there has been some criticism of this hypothesis since its proposal because it was induced only from the statistical analysis based on the receptor binding profile of atypical antipsychotic drugs (Meltzer et al., 1989), and Meltzer (1989) did not fully account for why the potent 5-HT_{2A} receptor antagonist with weaker D_2 receptor blockade exerts an antipsychotic action at a dose unlikely to produce EPS. Moreover, it has also been criticized because the classification of typical and atypical antipsychotic drugs as subjects of the analysis seems not definitive, but arbitrary.

The serotonin–dopamine hypothesis has been followed by a number of theories proposing that an interaction among multiple neurotransmitter systems may play an important role in the mechanism of action for atypical antipsychotic drugs. This may be attributed in part to the fact

Table 1. Binding potencies of antipsychotic drugs to 5-HT_{2A}/ D_2 receptors

Antipsychotic drugs	pK _i 5-HT _{2A}	pK _i D_2	5-HT _{2A} / D_2
Second-generation			
Clozapine	8.3	7.0	1.3
Risperidone	10.1	8.9	1.2
Olanzapine	8.7	7.8	0.9
Quetiapine	6.8	5.9	0.9
Ziprasidone	9.5	8.0	1.5
Zotepine	9.0	7.9	1.1
Aripiprazole ^a	8.1	9.1	−1.0
First-generation			
Haloperidol	7.7	9.0	−1.3
Chlorpromazine	8.7	8.5	0.2
Perphenazine	8.6	9.2	−0.6
Thioridazine	8.2	8.1	0.1
Sulpiride	4.5	6.4	−1.9
Pipamperone ^b	8.9	7.0	1.9

Notes: Data are expressed as pK_i ($= -\log K_i$) values (Meltzer et al., 1989; Leysen et al., 1993; Schotte et al., 1996; Shapiro et al., 2003). The difference in pK_i values between the 5-HT_{2A} and D_2 receptors indicates the magnitude of relatively greater affinity of an antipsychotic drug for 5-HT_{2A} receptors than for D_2 receptors

^aA partial D_2 agonist.

^bAn atypical antipsychotic with a low incidence of EPS.

that clozapine also has significant affinities for multiple receptors including 5-HT receptor subtypes other than 5-HT_{2A} receptors, D₁, D₄, α_1 -, α_2 -adrenergic, H₁ histaminergic and M₁ cholinergic receptors (Leysen et al., 1993; Schotte et al., 1996). Accordingly, olanzapine, which like clozapine displays a broad spectrum of affinities for multiple receptors, was suggested to have higher potential than other antipsychotic drugs to improve treatment-resistant schizophrenia (Bymaster et al., 1996).

PET imaging of SGAs

Recent advances in neuroimaging have made it possible to investigate the receptor occupancy in vivo of antipsychotic drugs in medicated patients. Positron emission tomography (PET) studies using specific radioactive ligands to D₂ receptors have demonstrated that for FGAs such as haloperidol, an almost 70% of D₂ receptor blockade is the optimal level for antipsychotic response, and occupancies greater than 80% are associated with increased incidence of EPS (Farde and Nordström, 1992; Seeman, 1995; Pilowsky et al., 1997).

In contrast, the basal ganglia D₂ receptor occupancy of clozapine at usual clinical doses has been reported to be lower than 50%. This is consistent with in vitro D₂ receptor binding of clozapine, suggesting its 'atypical' pharmacological features are distinct from other antipsychotic drugs.

Since the latter half of the 1990s, PET studies have revealed that the D₂ receptor occupancy of some SGAs is higher than that of clozapine (Fig. 1). At the doses clinically used, risperidone, olanzapine and ziprasidone have been shown to occupy D₂ receptors to a similar extent (60–90%) than haloperidol (Kapur and Remington 1996; Kapur et al., 1999; Nyberg et al., 1999; Mamo et al., 2004). In line with the in vitro binding potency of these drugs to D₂ receptors, PET data may account for a higher incidence of EPS as well as the prolactin elevation induced by these drugs at higher doses. Interestingly, aripiprazole at clinically relevant doses has demonstrated very high levels of D₂ occupancy in both normal volunteers and schizophrenic patients, while not producing EPS (Yokoi et al., 2002; Mamo et al., 2007).

Cortical 5-HT_{2A} occupancies of clozapine, risperidone, olanzapine and ziprasidone are

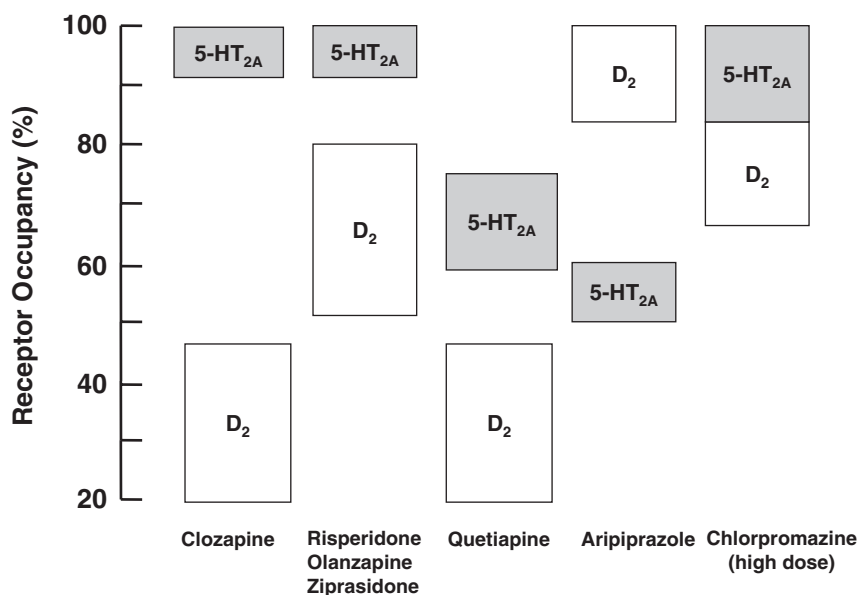


Fig. 1. PET data of in vivo occupancy of 5-HT_{2A} and D₂ receptors by antipsychotic drugs at usual clinical doses (Seeman, 1995; Trichard et al., 1998; Kapur et al., 1999; Nyberg et al., 1999; Gefvert et al., 2001; Mamo et al., 2007).

reported as very high (over 90%) (Kapur and Remington, 1996; Kapur et al., 1999; Nyberg et al., 1999; Mamo et al., 2004), while quetiapine and aripiprazole show slightly lower occupancy (60–70%) of 5-HT_{2A} receptors (Gefvert et al., 2001; Mamo et al., 2007) (Fig. 1). Thus, most SGAs, except for aripiprazole and amisulpride (a selective D_{2/3} antagonist), display relatively higher 5-HT_{2A} occupancy than D₂ occupancy, which is consistent with their in vitro profile of receptor binding. Taken together, Kapur and Remington (1996) initially suggested that a strong 5-HT_{2A} blockade might antagonize a relatively weaker D₂ blockade with low doses of risperidone to protect against EPS. However, as discussed later, they subsequently rejected any role for 5-HT_{2A} receptors in the action of SGAs (Kapur and Seeman, 2001).

While earlier studies suggested that clozapine might preferentially act on the D₂ receptor in the cortical and limbic areas rather than basal ganglia, some PET studies have shown relatively low D₂ receptor occupancy (30–50%) of clozapine in both the striatal and the extrastriatal regions (Pilowsky et al., 1997; Talvik et al., 2001). Another study reports that both haloperidol and clozapine achieve comparably high levels of D₂ receptor blockade in the temporal cortex (Xiberas et al., 2001). In contrast, more recent studies with high-resolution imaging demonstrate that clozapine produces preferential occupancy of D₂ receptors in the temporal cortex as opposed to the putamen of schizophrenic patients receiving clozapine (Gründer et al., 2006; Kessler et al., 2006). These conflicting results regarding the regional selectivity of clozapine to D₂ receptors may be due to the methodological limitation.

Some reports from neuroimaging studies have disputed the possible involvement of 5-HT_{2A} receptors in the mechanism of action of SGAs. Chlorpromazine at a high dose (700 mg/day) was reported to occupy most of the cortical 5-HT_{2A} receptors (Fig. 1; Trichard et al., 1998). There was also no correlation between clinical improvement and 5-HT_{2A} occupancy in clozapine-treated patients (Travis et al., 1998). Moreover, Knable et al. (1997) examined the relationship between D₂ occupancy and incidence of EPS in patients treated

with risperidone or haloperidol, and found that both risperidone and haloperidol at occupancy levels above 60% produced comparable incidence of EPS. These data do not support the hypothesis that 5-HT_{2A} receptor blockade may contribute to the superior efficacy of clozapine and SGAs in treatment of schizophrenia. Furthermore, the lack of significant antipsychotic efficacy of the selective 5-HT_{2A} antagonist M100,907 in a clinical trial has accelerated the arguments against the potential role of the 5-HT_{2A} receptor for clinical benefits of SGAs (Kapur and Seeman, 2001).

Controversy in the ‘fast-off’ theory

D₂ receptor occupancy of quetiapine, even at doses of 450–600 mg/day, is not more than 30% 12 h after the last dose (Kapur et al., 2000; Gefvert et al., 2001). However, Kapur et al. (2000) demonstrate that quetiapine as well as clozapine show higher D₂ occupancy (45–60%) within 2–3 h following its administration, subsequently declining rather rapidly. They consider that this phenomenon may be due to the loose binding property of these drugs to the D₂ receptor. Therefore, the injected radioactive ligand at its peak concentration is thought to displace antipsychotic drugs bound to the D₂ receptor, resulting in apparently low D₂ occupancy on the PET image (Seeman and Tallerico, 1999).

Taken together, Kapur and Seeman (2001) argue that the difference between typical and atypical antipsychotic drugs may be fully explained by the pharmacokinetics of their interaction with the D₂ receptor. This ‘fast-off’ theory has become a typical antithesis to the serotonin–dopamine hypothesis. It implies that the atypical antipsychotic effect can be produced by appropriate modulation of the D₂ receptor alone, while the blockade of the 5-HT_{2A} receptor and other receptors may be neither necessary nor sufficient. It therefore predicts that low doses of typical antipsychotic drugs such as haloperidol could achieve most, if not all, of the benefits of clozapine with regard to antipsychotic action and EPS. To date, the ‘fast-off’ binding of clozapine to the D₂ receptor has yet to be replicated in human subjects,

while PET studies on non-human primates (rhesus monkeys) have provided supportive data for the theory. Mukherjee et al. (2001) report that clozapine (9.7 mg/kg, s.c.) achieves approximately 70% occupancy of D₂ receptors 2–3 h after drug injection. Suhara et al. (2002) also demonstrate that D₂ receptor occupancy reaches more than 80% after 5.0 mg/kg of clozapine injection, and then rapidly decreases with a half-life of 7.2 h. Moreover, an isomer of clozapine having an equivalent affinity to clozapine on multiple receptors such as 5-HT_{1A}, 5-HT_{2A}, D₁, and M₁ but having a tenfold higher affinity at D_{2/3} receptors is found to facilitate catalepsy and prolactin elevation in rats (Kapur et al., 2002). The atypical action of clozapine has therefore been attributed to the binding property to the D₂ receptor alone, but not the additional interaction with 5-HT_{2A} receptors.

On the contrary, Meltzer et al. (2003) suggest that the ‘fast-off’ theory could only apply to clozapine and quetiapine but would not account for the pharmacological basis of other SGAs including olanzapine, risperidone and ziprasidone. When calculated from the data of Kapur and Seeman (2000), the time ($t_{1/2}$) for 50% displacement of clozapine or quetiapine from the cloned human D₂ receptor by excessive raclopride becomes only less than 1 min (Meltzer et al., 2003). However, the $t_{1/2}$ of two SGAs, olanzapine and sertindole, is 15–20 min, comparable to that of haloperidol and chlorpromazine. Moreover, risperidone is included in the ‘slow-off’ drug group, which is for the most part made up of the obviously typical FGAs (Seeman, 2002). It is therefore unlikely that the ‘fast-off’ D₂ binding alone could distinguish atypical antipsychotic drugs from typical ones. Furthermore, a PET study by Kessler et al. (2006) has demonstrated that the therapeutic effects of clozapine and quetiapine occur at D₂ occupancies that are lower than those seen with typical antipsychotic drugs, suggesting non-D₂ receptor-mediated mechanisms of action of atypical antipsychotic drugs.

It is evident that the ‘fast-off’ theory has provoked a critical question whether FGAs and SGAs are qualitatively different or not. Proposal of the theory corresponded to the same time that

some clinical studies began to suggest comparative effects of SGAs versus low-dose or low-potent FGAs with regard to clinical efficacy (Geddes and Harrison, 2000; Davis et al., 2003; Leucht et al., 2003; Moncrieff, 2003). However, as Meltzer et al. (2003) postulate, the ‘fast-off’ theory could hardly be generalized to the pharmacological model of SGAs. It predominantly focuses on lesser propensity of SGAs to produce EPS but has not fully addressed their neurocognitive effects. These effects have recently been demonstrated, as discussed later in this chapter, for their pharmacological basis (Meltzer and McGurk, 1999; Harvey and Keefe, 2001; Woodward et al., 2005). It should also be noted that most SGAs have been modelled on clozapine for development because this has been shown to be superior to typical antipsychotic drugs for improving treatment-resistant schizophrenia (Kane et al., 1988; Chakos et al., 2001; Davis et al., 2003; McEvoy et al., 2006), while a similar superior therapeutic effect of quetiapine, another ‘fast-off’ drug, has been inconclusive (Chakos et al., 2001; Davis et al., 2003; McEvoy et al., 2006). Therefore, there is a limitation to the ‘fast-off’ theory in the further development of new antipsychotic drugs.

5-HT_{1A} receptors and prefrontal dopamine

Many SGAs have significant affinities not only for 5-HT_{2A} receptors but also for other 5-HT receptor subtypes including 5-HT_{1A}, 5-HT_{2C}, 5-HT₆ and 5-HT₇ (Roth et al., 1992, 1994, 2004; Schotte et al., 1996). It is therefore possible that these 5-HT receptor subtypes may also be involved in the mechanisms of action of SGAs. Although physiological roles of the central 5-HT receptor subtypes have yet to be fully understood, the serotonin–dopamine interaction via 5-HT receptor subtypes has been thought to play an important role in the production of clinical effects of SGAs.

Some SGAs are found to have relatively high affinities for 5-HT_{1A} receptors. Clozapine, ziprasidone and quetiapine act on 5-HT_{1A} receptors as a partial agonist (Newman-Tancredi et al., 1998). Aripiprazole, a partial dopamine D_{2/3} agonist, also has a similar effect on 5-HT_{1A} receptors

(Shapiro et al., 2003). Consistent with these in vitro data, recent PET studies have demonstrated significant occupancy of 5-HT_{1A} receptors by clozapine and aripiprazole (Chou et al., 2003; Mamo et al., 2007). The 5-HT_{1A} receptor stimulation, in addition to the 5-HT_{2A} receptor blockade, may be the basis for clinical benefits of atypical antipsychotic drugs probably due to the functional interaction between 5-HT_{1A} and 5-HT_{2A} receptors (Araneda and Andrade, 1991; Wadenberg and Ahlenius, 1991; Millan, 2000).

While using in vivo microdialysis of experimental animals, acute administration of haloperidol increases dopamine release from the nerve terminal in the striatum and nucleus accumbens because of the blockade of presynaptic D₂ autoreceptors, while having no effect on that in the prefrontal cortex. In contrast, clozapine preferentially increases dopamine release in the prefrontal cortex to the subcortical regions (Moghaddam and Bunney, 1990; Volonté et al., 1997; Kuroki et al., 1999). Chronic treatment with clozapine does not develop tolerance to its ability to increase prefrontal dopamine release, suggesting augmentation of dopaminergic neurotransmission in the prefrontal cortex (Yamamoto and Cooperman, 1994; Youngren et al., 1994). Moreover, clozapine has also been shown to increase dopamine release in the prefrontal cortex of non-human primates in a region-specific manner (Youngren et al., 1999). In addition, most SGAs, including risperidone, olanzapine, quetiapine, ziprasidone and aripiprazole, have been demonstrated to facilitate dopamine release in the prefrontal cortex and hippocampus (Volonté et al., 1997; Kuroki et al., 1999; Rollema et al., 2000; Westerink et al., 2001; Ichikawa et al., 2002; Li et al., 2004). These effects may be the basis for the neurocognitive effects of SGAs on schizophrenia because SGAs have been thought to normalize the hypofrontality responsible for cognitive deficits of the condition (Meltzer and McGurk, 1999; Meltzer et al., 2003; Roth et al., 2004).

The ability of SGAs to increase prefrontal dopamine release has been related to their affinities for D₂, 5-HT_{2A} and also 5-HT_{1A} receptors (Kuroki et al., 1999; Rollema et al., 2000; Ichikawa et al., 2001a, b; Liégeois et al., 2002).

The effects of antipsychotic drugs to increase dopamine release in the prefrontal cortex relative to that in the nucleus accumbens are positively correlated with the difference between their pK_i values for 5-HT_{2A} and D₂ receptors (Kuroki et al., 1999), suggesting an involvement of the receptor profile of atypical antipsychotic drugs in their preference for the prefrontal cortex (Meltzer et al., 1989). Pretreatment with the selective 5-HT_{2A} antagonist M100,907 potentiates the ability of a low dose (0.1 mg/kg), but not a high dose (1.0 mg/kg), haloperidol to increase dopamine release in the prefrontal vortex, whereas it abolishes the effect of both doses of haloperidol on dopamine release in the nucleus accumbens (Andersson et al., 1995; Liégeois et al., 2002). Conversely, the 5-HT_{2A/2C} receptor agonist DOI attenuates the ability of clozapine to increase prefrontal dopamine release, and this effect is antagonized by M100,907 (Ichikawa et al., 2001a). These results suggest that the relatively higher ratio of 5-HT_{2A} to D₂ antagonism may contribute to the potentiation of antipsychotic-induced prefrontal dopamine release.

Rollema et al. (2000) and Ichikawa et al. (2001b) have further demonstrated that the selective 5-HT_{1A} antagonist WAY100,635 attenuates the ability of clozapine to increase dopamine release in the prefrontal cortex. WAY100,635 also inhibits the prefrontal dopamine-releasing potency of both ziprasidone and quetiapine that have high affinities for 5-HT_{1A} receptors (Rollema et al., 2000; Ichikawa et al., 2001b). Interestingly, WAY100,635 does attenuate the ability of both risperidone and olanzapine that lack a significant affinity for 5-HT_{1A} receptors (Ichikawa et al., 2001b). Moreover, M100,907 potentiates the ability of sulpride, a D₂ antagonist, to induce prefrontal dopamine release, and this effect is also prevented by pretreatment with WAY100,635 (Ichikawa et al., 2001b). Taken together, an interaction between potent 5-HT_{2A} antagonism and relatively weaker D₂ antagonism may activate 5-HT_{1A} receptor function to enhance dopamine release in the prefrontal cortex. This hypothesis is consistent with a recent study showing knockout mice for 5-HT_{1A} receptor gene to diminish the atypical antipsychotic-induced dopamine release in the

prefrontal cortex after systemic and local administration (Díaz-Mataix et al., 2005). Activation of prefrontal 5-HT_{1A} receptors by atypical antipsychotic drugs may enhance the activity of dopamine neurons in the ventral tegmental area (VTA) and then facilitate dopamine release from the nerve terminal in the prefrontal cortex (Díaz-Mataix et al., 2005).

While aripiprazole, a partial agonist for D₂ receptors, has considerable binding affinity for both 5-HT_{1A} and 5-HT_{2A} receptors (Shapiro et al., 2003), the effect of systemic administration of aripiprazole on dopamine release in the prefrontal cortex has been equivocal (Semba et al., 1995; Jordan et al., 2004; Li et al., 2004). This may be attributable in part to the agonistic action of aripiprazole on presynaptic D₂ autoreceptors that regulate dopamine release from the nerve terminal in the prefrontal cortex. We examined whether aripiprazole affects prefrontal dopamine release when given locally into dopamine neurons of the VTA using microdialysis with dual probe implantation of awake, freely moving rats (Kuroki et al., 2007). When applied locally into the VTA, aripiprazole produced a significant decrease in dopamine release in the prefrontal cortex that receives dopaminergic projection from the VTA (Fig. 2A). The effect of aripiprazole resembled that of quinpirole, a full D₂ receptor agonist, but not of raclopride, a D₂ antagonist (Fig. 2B). When applied directly into the prefrontal cortex, aripiprazole increased prefrontal dopamine release, and this effect was completely abolished by pretreatment with WAY100,635 (Fig. 2C). Pretreatment with WAY100,635 also attenuated the increase in prefrontal dopamine release provoked by locally administered clozapine (Fig. 2D). These results suggest that aripiprazole, like clozapine, may enhance dopaminergic activity of the local circuitry in the prefrontal cortex due to activation of 5-HT_{1A} receptors. This is consistent with a recent study showing a lack of aripiprazole-induced dopamine release in the prefrontal cortex of 5-HT_{1A} knockout mice (Bortolozzi et al., 2007).

The prefrontal dopaminergic activation by SGAs, as discussed in preceding paragraphs, may contribute to the effects of these drugs on cognitive symptoms related to the prefrontal cortex of

schizophrenics (Meltzer and McGurk, 1999; Harvey and Keefe, 2001; Woodward et al., 2005). In this regard, Sumiyoshi et al. (2001) reported that tandospirone, a 5-HT_{1A} partial agonist, added to typical antipsychotic treatment improved executive function, verbal learning and memory in schizophrenia. They subsequently showed buspirone, a 5-HT_{1A} partial agonist, enhanced attention, but not other cognitive domains, in schizophrenic patients who had been treated with atypical antipsychotic drugs (Sumiyoshi et al., 2007). Clearly, further investigation needs to elucidate the role of 5-HT_{1A} receptors in the cognitive action of SGAs.

Interactions with 5-HT_{2C}, 5-HT₆ and 5-HT₇ receptors

Clozapine, olanzapine, ziprasidone and aripiprazole have comparable affinities ($K_i < 10$ nM) for 5-HT_{2C} receptors to 5-HT_{2A} receptors, while affinities of quetiapine and risperidone for 5-HT_{2C} receptors are lower than those for 5-HT_{2A} receptors (Roth et al., 1992; Schotte et al., 1996; Shapiro et al., 2003). Roth et al. (1992) have suggested that a relatively higher binding potency of the 5-HT_{2C} receptor could not differentiate typical and atypical antipsychotic drugs. It is therefore unlikely that binding potency to 5-HT_{2C} receptors plays a major role in the mechanism of action of SGAs. However, 5-HT_{2A} and 5-HT_{2C} receptors are thought to interact reciprocally to modulate the activity of the mesolimbic and mesocortical dopaminergic pathways. The 5-HT_{2C} receptor may tonically inhibit dopamine release in the prefrontal cortex and the nucleus accumbens, whereas the 5-HT_{2A} receptor may stimulate it in a phasic manner (Millan et al., 1998; Gobert and Millan, 1999; Kuroki et al., 2003). This functional interaction between 5-HT_{2A} and 5-HT_{2C} receptors may contribute, at least in part, to the difference in clinical profiles among SGAs, which vary in their 5-HT_{2A}/5-HT_{2C} ratios. For example, 5-HT_{2C} receptor antagonism has been related to weight gain, one of the frequent and critical adverse effects of SGAs. Evidence has been based on the observation that knockout mice for the 5-HT_{2C}

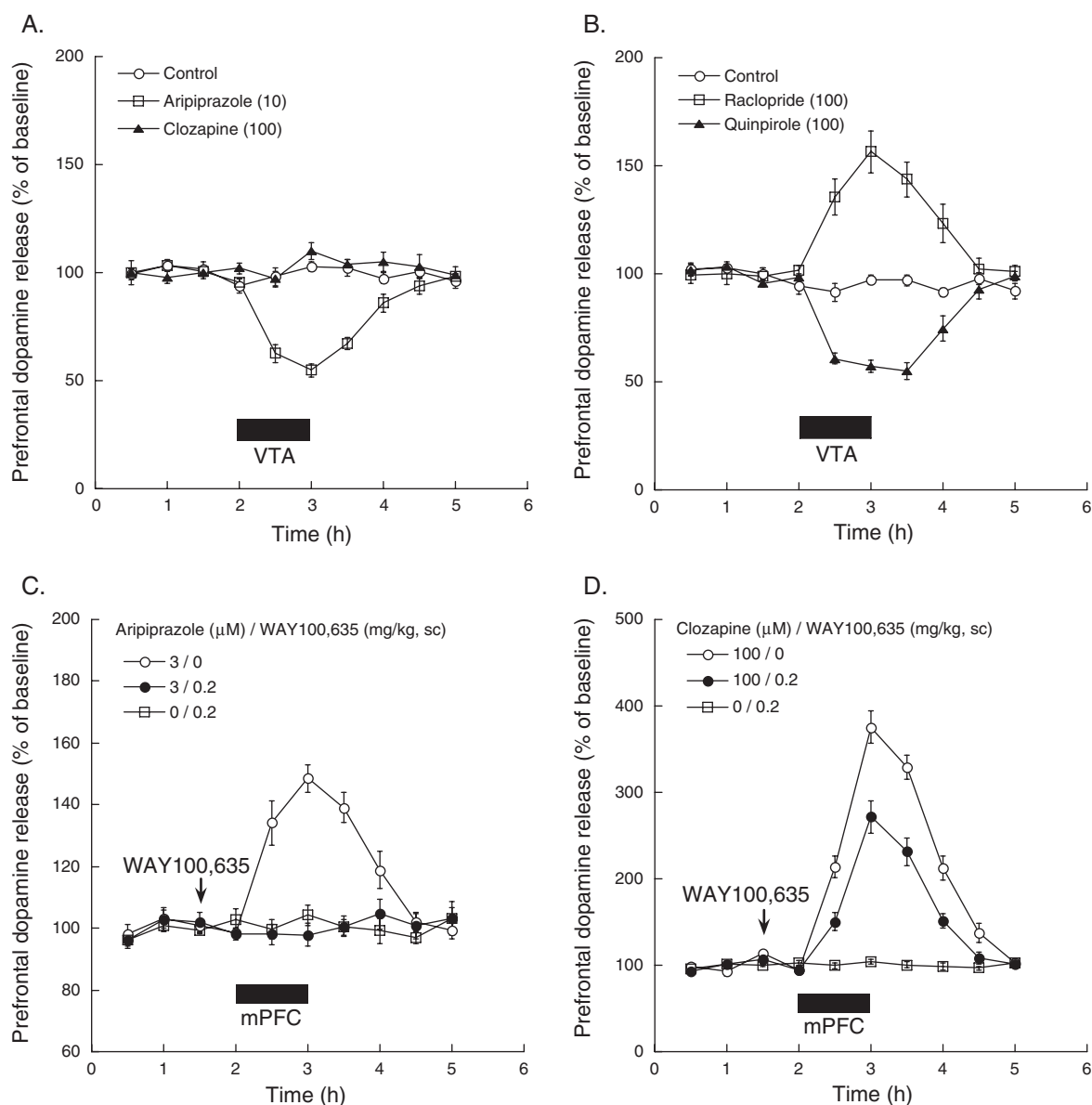


Fig. 2. Dual effects of the partial D_2 agonist aripiprazole on dopamine release in the medial prefrontal cortex, as determined by in vivo microdialysis of awake, freely moving rats (Kuroki et al., 2007). When applied locally into the ventral tegmental area (VTA), aripiprazole (10 μ M) produced a significant decrease in dopamine release in the prefrontal cortex (mPFC) (A). This effect closely resembled that of quinpirole (100 μ M), a full D_2 agonist (B), but not raclopride (100 μ M), a D_2 antagonist. Clozapine (100 μ M) had no effect on prefrontal dopamine release following the local perfusion into the VTA. When applied directly into the mPFC, both aripiprazole and clozapine increased dopamine release (C and D). These effects were abolished or attenuated by pretreatment with WAY100,635 (0.2 mg/kg, s.c.), a 5-HT_{1A} antagonist, 60 min prior to perfusion of each antipsychotic drug. Data are mean \pm S.E.M., expressed as percentage of predrug baseline (100%). The time-dependent effects of drugs were analysed by repeated measure ANOVA compared to control group of rats. Shaded columns indicate the period of local perfusion with each drug via the dialysis probe.

receptor gene develop obesity (Nonogaki et al., 2003) and that antipsychotic-induced weight gain is reported to be associated with a polymorphism (C759T) of the promoter region of the 5-HT_{2C} receptor gene in schizophrenic patients (Reynolds et al., 2002).

The 5-HT₆ and 5-HT₇ receptors may be involved in the mechanism of motor effects of SGAs because clozapine shows high binding potencies ($K_i < 10$ nM) to both receptors, and the striatum contains abundant mRNAs of the 5-HT₆ receptor (Roth et al., 1994). Chronic treatment with clozapine is reported to decrease 5-HT₆ receptor mRNA levels in the hippocampus (Frederick and Meador-Woodruff, 1999). In addition, a pharmacogenetic study reported an association of a 5-HT₆ receptor gene polymorphism (C267T) with a clinical response to clozapine in schizophrenic patients (Yu et al., 1999), although this finding was not replicated later. Among SGAs, olanzapine and zotepine have high affinities for the 5-HT₆ receptor, while the binding potencies of risperidone and zotepine to the 5-HT₇ receptor are high (Roth et al., 1994). However, some FGAs (chlorpromazine and thioridazine) have high affinities for the 5-HT₆ receptor, and other FGAs (fluphenazine and pimozide) bind to the 5-HT₇ receptor potently (Roth et al., 1994). Therefore, high affinities for either receptor may not be specific to the pharmacological features of SGAs. Knowledge about the role of 5-HT₆ and 5-HT₇ receptors in the action of antipsychotic drugs is still scanty. Chronic administration of antipsychotic drugs such as haloperidol, chlorpromazine, olanzapine, risperidone and clozapine for 2 weeks had no effect on the binding of [¹²⁵I]SB-258585, a selective 5-HT₆ antagonist, in the rat brain (East et al., 2002). Acute and chronic administration of the 5-HT₆ antagonist SB-271046 was shown to affect the activity of the midbrain dopamine neurons, its pattern differed from that of either typical or atypical antipsychotic drugs (Minabe et al., 2004). More recently, the 5-HT₆ antagonist SB-399885 is reported to potentiate both haloperidol-induced and risperidone-induced dopamine release in the prefrontal cortex or hippocampus, suggesting a possible therapeutic role of 5-HT₆ receptor antagonism to enhance cognitive function in schizophrenia (Li et al., 2007).

Towards future research to elucidate the role of serotonin–dopamine interaction in SGAs

For the past two decades, drug discovery research has vigorously attempted to develop a novel antipsychotic drug modelled on clozapine. The serotonin–dopamine hypothesis is the most important landmark, and has contributed to the development of a number of SGAs. Nevertheless, to date, an antipsychotic drug having comparable or superior effects on treatment-resistant schizophrenia has yet to be found (Chakos et al., 2001; Davis et al., 2003; McEvoy et al., 2006). Many attempts have unexpectedly failed to develop more effective antipsychotic drugs by targeting the specific single receptor responsible for clozapine's effects. Therefore, some investigators have recently come to consider the fact that clozapine has a highly complex pharmacological profile with high affinity to numerous receptors, but not with selectivity to any single receptor or molecule. For example, Roth et al. (2004) comment in a review titled *Magic Shotguns Versus Magic Bullets* that designing selectively non-selective drugs that interact with several molecular targets, that is 'magic shotguns', will lead to new, more effective medications for schizophrenia. Their proposal is based on the observation that 5-HT_{2A} and many other receptors have been demonstrated to modulate glutamatergic and dopaminergic neurotransmission in the prefrontal cortex, their interaction may be the basis for cognitive function. The success of aripiprazole, a partial D₂ agonist with 5-HT_{1A} agonism and 5-HT_{2A} antagonism, in efficacy and tolerability in treatment of schizophrenia has also stimulated further development of a partial agonist or modulator of several receptors as a more effective antipsychotic drug. Further research is needed to elucidate dopamine–serotonin interactions via multiple 5-HT receptor subtypes and determine how to utilize this information for designing newer antipsychotic agents. More complex agents such as clozapine may have greater potential for interacting at various elements of the circuitry that underlies the multiple deficits of schizophrenia.

Abbreviations

EPS	extrapyramidal symptoms
FGAs	first-generation antipsychotic drugs
K_i	dissociation constant
SGAs	second-generation antipsychotic drugs
5-HT	serotonin
VTA	ventral tegmental area

Acknowledgements

The authors acknowledge fruitful discussion with Junji Ichikawa, MD. The research reported here was supported in part by KAKENHI (17591219) and a grant from Otsuka Pharmaceuticals.

References

- Andersson, J.L., Nomikos, G.G., Marcus, M., Hertel, P., Mathé, J.M. and Svensson, T.H. (1995) Risperidone potentiates the stimulatory effects of raclopride on neuronal activity and dopamine release selectively in the mesolimbic dopaminergic systems. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 352: 374–385.
- Araneda, R. and Andrade, R. (1991) 5-Hydroxytryptamine₂ and 5-hydroxytryptamine_{1A} receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience*, 40: 399–412.
- Bortolozzi, A., Díaz-Mataix, L., Toth, M., Celada, P. and Artigas, F. (2007) In vivo actions of aripiprazole on serotonergic and dopaminergic systems in rodent brain. *Psychopharmacology*, 191: 745–758.
- Bymaster, F.P., Calligaro, D.O., Falcone, J.F., Marsh, R.D., Moore, N.A., Tye, N.C., Seaman, P. and Wong, D.T. (1996) Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology*, 14: 87–96.
- Chakos, M., Lieberman, J., Hoffman, E., Bradford, D. and Sheitman, B. (2001) Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. *Am. J. Psychiatry*, 158: 518–526.
- Chou, Y.H., Halldin, C. and Farde, L. (2003) Occupancy of 5-HT_{1A} receptors by clozapine in the primate brain: a PET study. *Psychopharmacology*, 166: 234–240.
- Davis, J.M., Chen, N. and Glick, I.D. (2003) A meta-analysis of the efficacy of second-generation antipsychotics. *Arch. Gen. Psychiatry*, 60: 553–564.
- Díaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25: 10831–10843.
- East, S.Z., Burnet, P.W.J., Leslie, R.A., Roberts, J.C. and Harrison, P.J. (2002) 5-HT₆ receptor binding sites in schizophrenia and following antipsychotic drug administration: autoradiographic studies with [¹²⁵I]SB-258585. *Synapse*, 45: 191–199.
- Farde, L. and Nordström, A.-L. (1992) PET analysis indicates atypical central dopamine receptor occupancy in clozapine-treated patients. *Br. J. Psychiatry*, 160(Suppl. 17): 30–33.
- Frederick, J.A. and Meador-Woodruff, J.H. (1999) Effects of clozapine and haloperidol on 5-HT₆ receptor mRNA levels in rat brain. *Schizophr. Res.*, 38: 7–12.
- Geddes, J. and Harrison, P. (2000) Atypical antipsychotics in the treatment of schizophrenia: systemic overview and meta-regression analysis. *BMJ*, 321: 1371–1376.
- Gefvert, O., Lundberg, T., Wieselgren, I.-M., Bergström, M., Langström, B., Wiesel, F. and Lindström, L. (2001) D₂ and 5-HT_{2A} receptor occupancy of different doses of quetiapine in schizophrenia: a PET study. *Eur. Neuropsychopharmacol.*, 11: 105–110.
- Gobert, A. and Millan, M.J. (1999) Serotonin (5-HT)_{2A} receptor activation enhances dialysis levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology*, 38: 315–317.
- Gründer, G., Landvogt, C., Vernaleken, I., Buchholz, H.-G., Ondracek, J., Siessmeier, T., Härtter, S., Schreckenberger, M., Stoeter, P., Hiemke, C., Rösch, F., Wong, D.F. and Bartenstein, P. (2006) The striatal and extrastriatal D_{2/3} receptor-binding profile of clozapine in patients with schizophrenia. *Neuropsychopharmacology*, 31: 1027–1035.
- Harvey, P.D. and Keefe, R.S.E. (2001) Studies of cognitive change in patients with schizophrenia following novel antipsychotic treatment. *Am. J. Psychiatry*, 158: 176–184.
- Ichikawa, J., Dai, J. and Meltzer, H.Y. (2001a) DOI, a 5-HT_{2A/2C} receptor agonist, attenuates clozapine-induced cortical dopamine release. *Brain Res.*, 907: 151–155.
- Ichikawa, J., Ishii, H., Bonaccorso, S., Fowler, W.L., O'Laughlin, I.A. and Meltzer, H.Y. (2001b) 5-HT_{2A} and D₂ receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.*, 76: 1521–1531.
- Ichikawa, J., Li, Z., Dai, J. and Meltzer, H.Y. (2002) Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: role of 5-HT_{1A} receptor agonism. *Brain Res.*, 956: 349–357.
- Jordan, S., Koprivica, V., Dunn, R., Tottori, K., Kikuchi, T. and Altar, C.A. (2004) In vivo effects of aripiprazole on cortical and striatal dopaminergic and serotonergic function. *Eur. J. Pharmacol.*, 483: 45–53.
- Kane, J., Hönigfeld, G., Singer, J. and Meltzer, H.Y. The Clozaril Collaborative Study Group. (1988) Clozapine for the treatment-resistant schizophrenic. *Arch. Gen. Psychiatry*, 45: 789–796.

- Kapur, S., McClelland, R.A., VanderSpek, S.C., Wandenbergh, M.L., Baker, G., Nobrega, J., Sipursky, R.B. and Seeman, P. (2002) Increasing D₂ affinity results in the loss of clozapine's atypical antipsychotic action. *Neuroreport*, 13: 831–835.
- Kapur, S. and Remington, G. (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. *Am. J. Psychiatry*, 153: 466–476.
- Kapur, S. and Seeman, P. (2000) Antipsychotic agents differ in how fast they come off dopamine D₂ receptors. Implications for atypical antipsychotic action. *J. Psychiatry Neurosci.*, 25: 161–166.
- Kapur, S. and Seeman, P. (2001) Does fast dissociation from the dopamine D₂ receptor explain the action of atypical antipsychotics?: a new hypothesis. *Am. J. Psychiatry*, 158: 360–369.
- Kapur, S., Zipursky, R., Jones, C., Shammi, C.S., Remington, G. and Seeman, P. (2000) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D₂ receptor occupancy. *Arch. Gen. Psychiatry*, 57: 553–559.
- Kapur, S., Zipursky, R.B. and Remington, G. (1999) Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am. J. Psychiatry*, 156: 286–293.
- Kessler, R.M., Ansari, M.S., Riccardi, P., Li, R., Jayathilake, K., Dawant, B. and Meltzer, H.Y. (2006) Occupancy of striatal and extrastriatal dopamine D₂ receptors by clozapine and quetiapine. *Neuropsychopharmacology*, 31: 1991–2001.
- Knable, M.B., Heinz, A., Raedler, T. and Weinberger, D.R. (1997) Extrapyramidal side effects with risperidone and haloperidol at comparable D₂ receptor occupancy levels. *Psychiatric Res.*, 75: 91–101.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (1999) Effects of antipsychotic drugs on extracellular dopamine levels in rat prefrontal cortex and nucleus accumbens. *J. Pharmacol. Exp. Ther.*, 288: 774–781.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (2003) 5-HT_{2A} receptor stimulation by DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Brain Res.*, 972: 216–221.
- Kuroki, T., Nakahara, T., Nagao, N., Motomura, K., Hashimoto, K. and Kanba, S. (2007) Dual effects of aripiprazole on prefrontal dopamine release. *Bull. Jpn Soc. Neurochem.*, 46: 493.
- Leucht, S., Pitschel-Walz, G., Engel, R.R. and Kissling, W. (2002) Amisulpride, an unusual "atypical" antipsychotic: a meta-analysis of randomized controlled trials. *Am. J. Psychiatry*, 159: 180–190.
- Leucht, S., Wahlbeck, K., Hamann, J. and Kissling, W. (2003) New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. *Lancet*, 361: 1581–1589.
- Leysen, J.E., Janssen, P.M.F., Schotte, A., Luyten, W.H.M.L. and Megens, A.A.H.P. (1993) Interactions of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5-HT₂ receptors. *Psychopharmacology*, 112: S40–S54.
- Li, Z., Huang, M., Prus, A.J., Dai, J. and Meltzer, H.Y. (2007) 5-HT₆ receptor antagonist SB-399885 potentiates haloperidol and risperidone-induced dopamine efflux in the medial prefrontal cortex or hippocampus. *Brain Res.*, 1134: 70–78.
- Li, Z., Ichikawa, J., Dai, J. and Meltzer, H.Y. (2004) Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. *Eur. J. Pharmacol.*, 493: 75–83.
- Liégeois, J.F., Ichikawa, J. and Meltzer, H.Y. (2002) 5-HT_{2A} receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Res.*, 947: 157–165.
- Lohr, J.B. and Braff, D.L. (2003) The value of referring to recently introduced antipsychotics as "second generation". *Am. J. Psychiatry*, 160: 1371–1372.
- Mamo, D., Graff, A., Mizrahi, R., Shammi, C.M., Romeyer, F. and Kapur, S. (2007) Differential effects of aripiprazole on D₂, 5-HT₂, and 5-HT_{1A} receptor occupancy in patients with schizophrenia: a triple trace PET study. *Am. J. Psychiatry*, 164: 1411–1417.
- Mamo, D., Kapur, S., Shammi, C.M., Papathodorou, G., Mann, S., Therrien, F. and Remington, G. (2004) A PET study of dopamine D₂ and serotonin 5-HT₂ receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone. *Am. J. Psychiatry*, 161: 818–825.
- Matsubara, S. and Meltzer, H.Y. (1989) Effect of typical and atypical antipsychotic drugs on 5-HT₂ receptor density in rat cerebral cortex. *Life Sci.*, 45: 1397–1406.
- McEvoy, J.P., Lieberman, J.A., Stroup, T.S., Davis, S.M., Meltzer, H.Y., Rosenheck, R.A., Swartz, M.S., Perkins, D.O., Keefe, R.S., Davis, C.E., Severe, J. and Hsiao, J.K. CATIE Investigators. (2006) Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am. J. Psychiatry*, 163: 600–610.
- Meltzer, H.Y. (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology*, 99: S18–S27.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159–1172.
- Meltzer, H.Y., Matsubara, S. and Lee, J. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251: 238–246.
- Meltzer, H.Y. and McGurk, S.R. (1999) The effects of clozapine, risperidone and olanzapine on cognitive function in schizophrenia. *Schizophr. Bull.*, 25: 233–256.
- Millan, M.J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J. Pharmacol. Exp. Ther.*, 295: 853–861.
- Millan, M.J., Dekeyne, A. and Gobert, A. (1998) Serotonin (5-HT)_{2C} receptors tonically inhibit dopamine (DA) and

- noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. *Neuropharmacology*, 37: 953–955.
- Minabe, Y., Shirayama, Y., Hashimoto, K., Routledge, C., Hagan, J.J. and Ashby, C.R., Jr. (2004) Effect of the acute and chronic administration of the selective 5-HT₆ receptor antagonist SB-271046 on the activity of midbrain dopamine neurons in rats: an in vivo electrophysiological study. *Synapse*, 52: 20–28.
- Moghaddam, B. and Bunney, B.S. (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. *J. Neurochem.*, 54: 1755–1760.
- Moncrieff, J. (2003) Clozapine vs. conventional antipsychotic drugs for treatment-resistant schizophrenia: a re-examination. *Br. J. Psychiatry*, 183: 161–166.
- Mukherjee, J., Christian, B.T., Narayanan, T.K., Shi, B. and Mantil, J. (2001) Evaluation of dopamine D-2 receptor occupancy by clozapine, risperidone, and haloperidol in vivo in the rodent and nonhuman primate brain using ¹⁸F-fallypride. *Neuropsychopharmacology*, 25: 476–488.
- Newman-Tancredi, A., Gavaudan, S., Conte, C., Chaput, C., Touzard, M., Verrielle, L., Audinot, V. and Millan, M.J. (1998) Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [³⁵S]GTPγS binding study. *Eur. J. Pharmacol.*, 355: 245–256.
- Nonogaki, K., Abdallah, L., Goulding, E.H., Bonasera, S.J. and Tecott, L.H. (2003) Hyperactivity and reduced energy cost of physical activity in serotonin 5-HT_{2C} receptor mutant mice. *Diabetes*, 52: 315–320.
- Nyberg, S., Eriksson, B., Oenstierna, G., Halldin, C. and Farde, L. (1999) Suggested minimal effective dose of risperidone based on PET-measured D₂ and 5-HT_{2A} receptor occupancy in schizophrenic patients. *Am. J. Psychiatry*, 156: 869–875.
- Pilowsky, L.S., Mulligan, R.S., Acton, P.D., Ell, P.J., Costa, D.C. and Kerwin, R.W. (1997) Limbic selectivity of clozapine. *Lancet*, 350: 490–491.
- Reynolds, G.P., Zhang, Z.-L. and Zhang, X.-B. (2002) Association of antipsychotic drug-induced weight gain with 5-HT_{2C} receptor gene polymorphism. *Lancet*, 359: 2086–2087.
- Rollema, H., Lu, Y., Schmidt, A.W., Sprouse, J.S. and Zorn, S.H. (2000) 5-HT_{1A} receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biol. Psychiatry*, 48: 229–237.
- Roth, B.L., Ciaranello, R.D. and Meltzer, H.Y. (1992) Binding of typical and atypical antipsychotic agents to transiently expressed 5-HT_{1C} receptors. *J. Pharmacol. Exp. Ther.*, 260: 1361–1365.
- Roth, B.L., Craig, S.E., Choudhary, M.S., Uluer, A., Monsma, F.J., Jr., Shen, Y., Meltzer, H.Y. and Sibley, D.R. (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.*, 268: 1403–1410.
- Roth, B.L., Scheffler, D.J. and Kroeze, W.K. (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.*, 3: 353–359.
- Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., Gompel, P.V., Lesage, A.S., De Loore, K. and Leysen, J.E. (1996) Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology*, 124: 57–73.
- Seeman, P. (1995) Dopamine receptors: clinical correlates. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 295–302.
- Seeman, P. (2002) Atypical antipsychotics: mechanism of action. *Can. J. Psychiatry*, 47: 27–38.
- Seeman, P. and Tallerico, T. (1999) Rapid release of antipsychotic drugs from dopamine D2 receptors: an explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. *Am. J. Psychiatry*, 156: 876–884.
- Semba, J., Watanabe, A., Kito, S. and Toru, M. (1995) Behavioural and neurochemical effects of OPC-14597, a novel antipsychotic drug, on dopaminergic mechanisms in rat brain. *Neuropharmacology*, 34: 785–791.
- Shapiro, D.A., Renock, S., Arrington, E., Chiodo, L.A., Liu, L.X., Sibley, D.R., Roth, B.L. and Mailman, R. (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology*, 28: 1400–1411.
- Sokoloff, P., Giros, B., Martres, M.P., Bouthenet, M.-L. and Schwartz, J.C. (1990) Molecular cloning and characterization of a novel dopamine receptor D₃ as a target for neuroleptics. *Nature*, 347: 146–151.
- Suhara, T., Okauchi, T., Sudo, Y., Takano, A., Kawabe, K., Maeda, J. and Kapur, S. (2002) Clozapine can induce high dopamine D₂ receptor occupancy. *Psychopharmacology*, 160: 107–112.
- Sumiyoshi, T., Matsui, M., Nohara, S., Yamashita, I., Kurachi, M., Sumiyoshi, C., Jayatilake, K. and Meltzer, H.Y. (2001) Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *Am. J. Psychiatry*, 158: 1722–1725.
- Sumiyoshi, T., Park, S., Jayatilake, K., Roy, A., Ertugrul, A. and Meltzer, H.Y. (2007) Effect of buspirone, a serotonin_{1A} partial agonist, on cognitive function in schizophrenia: a randomized, double-blind, placebo-controlled study. *Schizophr. Res.*, 95: 158–168.
- Talvik, M., Nordström, A.-L., Nyberg, S., Olsson, H., Halldin, C. and Farde, L. (2001) No support for regional selectivity in clozapine-treated patients: a PET study with [¹¹C]raclopride and [¹¹C]FLB457. *Am. J. Psychiatry*, 158: 926–930.
- Travis, M.J., Busatto, G.F., Pilowsky, L.S., Mulligan, R., Acton, P.D., Gacinovic, S., Mertens, J., Terrière, D., Costa, D.C., Ell, P.J. and Kerwin, R.W. (1998) 5-HT_{2A} receptor blockade in patients with schizophrenia treated with risperidone or clozapine: a SPECT study using the novel 5-HT_{2A} ligand ¹²³I-5-I-R-91150. *Br. J. Psychiatry*, 173: 236–241.
- Trichard, C., Paillere-Martinot, M.-L., Attar-Levy, D., Recassens, C., Monnet, F. and Martinot, J.-L. (1998) Binding

- of antipsychotic drugs to cortical 5-HT_{2A} receptors: a PET study of chlorpromazine, clozapine and amisulpride in schizophrenic patients. *Am. J. Psychiatry*, 155: 505–508.
- Volonté, M., Monferini, E., Cerutti, M., Fodritto, F. and Borsini, F. (1997) BIMG 80, a novel potential antipsychotic drug: evidence for multireceptor actions and preferential release of dopamine in prefrontal cortex. *J. Neurochem.*, 69: 182–190.
- Wadenberg, M.L. and Ahlenius, S. (1991) Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. *J. Neural. Transm. Gen. Sect.*, 83: 43–53.
- Westerink, B.H.C., Kawahara, Y., De Boer, P., Geels, C., De Vries, J.B., Wikstrom, H.V., Van Kalker, A., Van Vliet, B., Kruse, C.G. and Long, S.K. (2001) Antipsychotic drugs classified by their effects on the release of dopamine and noradrenaline in the prefrontal cortex and striatum. *Eur. J. Pharmacol.*, 412: 127–138.
- Woodward, N.D., Purdon, S.E., Meltzer, H.Y. and Zald, D.H. (2005) A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. *Int. J. Neuropsychopharmacol.*, 8: 457–472.
- Xiberras, X., Martinot, J.L., Mallet, L., Artiges, E., Loc'h, C., Maziere, B. and Paillere-Martinot, M.L. (2001) Extrastriatal and striatal D₂ dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. *Br. J. Psychiatry*, 179: 503–508.
- Yamamoto, B.K. and Cooperman, M.A. (1994) Differential effects of chronic antipsychotic drug treatment on extracellular glutamate and dopamine concentrations. *J. Neurosci.*, 14: 4159–4166.
- Yokoi, F., Grunder, G., Biziere, K., Stephane, M., Dogan, A.S., Dannals, H., Ravert, H., Suri, A., Bramer, S. and Wong, D.F. (2002) Dopamine D₂ and D₃ receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [¹¹C]raclopride. *Neuropsychopharmacology*, 27: 248–259.
- Youngren, K.D., Inglis, F.M., Pivrotto, P.J., Jedema, H.P., Bradberry, C.W., Goldman-Rakic, P.S., Roth, R.H. and Moghaddam, B. (1999) Clozapine preferentially increases dopamine release in the rhesus monkey prefrontal cortex compared with the caudate nucleus. *Neuropsychopharmacology*, 20: 403–412.
- Youngren, K.D., Moghaddam, B., Bunney, B.S. and Roth, R.H. (1994) Preferential activation of dopamine overflow in prefrontal cortex produced by chronic clozapine treatment. *Neurosci. Lett.*, 165: 41–44.
- Yu, Y., Tsai, S.-J., Lin, C.-H., Hsu, C.-P., Yang, K.-H. and Hong, C.-J. (1999) Serotonin-6 receptor variant (C267T) and clinical response to clozapine. *Neuroreport*, 10: 1231–1233.

CHAPTER 11

Serotonin–dopamine interactions: implications for the design of novel therapeutic agents for psychiatric disorders

Martyn D. Wood^{1,*} and Paul B. Wren²

¹*Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline, Harlow, CM19 5AW, UK*

²*Psychiatry Centre of Excellence for Drug Discovery, Medicines Research Centre, 37135, Verona, Italy*

Abstract: A close interplay exists between the serotonergic and dopaminergic neuronal systems both at the anatomical and functional level. It has long been known, at least in mammals, that the central serotonergic system modulates the activity of dopaminergic neurons in both the nigrostriatal pathway and ventral tegmental area. Since the discovery that reserpine and amphetamine induce symptoms in man that resemble those associated with depression and schizophrenia respectively, much attention has focussed on the development of drugs which affect the serotonergic and dopaminergic systems in psychiatric disorders. In this chapter, we will review some of the current research strategies targeting this neurotransmitter interaction that have driven compounds into clinical development in an attempt to provide more effective and safe medicines for such debilitating diseases.

Keywords: antipsychotic drugs; antidepressant drugs; schizophrenia; depression; receptors; transporters; MAO

Introduction

Changes in brain dopamine (DA) and serotonin (5-HT) neuronal systems have long been implicated in the aetiology and treatment of psychiatric disorders including schizophrenia and depression. In the 1960s, it was noted that in amphetamine addicts and depressed patients treated with amphetamine, many would develop a paranoid psychosis which was clinically indistinguishable from paranoid schizophrenia (Bell, 1965). Cocaine psychosis also has a number of similarities to paranoid schizophrenia and treatment with both cocaine and

amphetamine was associated with hyper-stimulation of DA receptors. It was later noted that hallucinogenic agents are agonists at the 5-HT_{2A} receptor, and that their behavioural effects resemble the positive symptoms of schizophrenia (Geyer, 1998; Vollenweider et al., 1998). In the 1950s it was observed that the rauwolfia alkaloid reserpine, which was used to treat hypertension, was found to cause a serious depressive state which was indistinguishable from endogenous depression. Unrelated to this observation, in 1957 it was found that the drugs isoniazid and iproniazid, monoamine oxidase inhibitors (MAOI) which were being used to treat tuberculosis, and the phenothiazine imipramine, which was undergoing trials as a neuroleptic, all produced an elevation of mood. When reserpine was subsequently found to lower brain

*Corresponding author. Tel.: +0044 01279-622247;
Fax: +0044 01279-622230; E-mail: Martyn.Wood@gsk.com

noradrenaline concentrations, and imipramine and the MAOIs were shown to increase brain noradrenaline, it was suggested that depression was associated with a decreased noradrenergic activity (Bunney and Davis, 1965; Schildkraut, 1965). With the latter realisation that these drugs also affected 5-HT functioning, this theory was revised to include serotonergic systems (Lapin and Oxenkrug, 1969). Furthermore, reserpine lowers brain DA concentrations and many tricyclic antidepressants also block DA uptake, which has led to further revision of the monoamine theory of depression.

In addition to emerging evidence that drugs used to treat psychiatric disorders affected monoamine nervous systems, substantial evidence to support a close interaction between the monoaminergic nervous systems and, in particular, the dopaminergic and serotonergic neuronal systems, was also becoming apparent. Thus, drugs which affected one system had effects on the other.

Serotonin/dopamine systems

There are three major DA systems in the brain (Wolf et al., 1987). The nigrostriatal pathway originates from cell bodies, which reside in the substantia nigra pars compacta (SNc) and project to the dorsal striatum (caudate-putamen). Degeneration of these neurons results in the motor deficits of Parkinson's disease. The mesolimbic pathway originates in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (NAc). The mesocortical pathway also originates in the VTA but projects to the prefrontal cortex where it is thought to regulate cognitive processes such as attention and working memory.

5-HT-containing neurons originating from the medial and dorsal raphe nuclei innervate both the substantia nigra and the VTA. Serotonergic terminals make direct synaptic contacts with DA-containing neurons in the SNc and VTA (Herve et al., 1987). In addition, terminal areas of the SNc and VTA receive an input from serotonergic neurons originating in the raphe nuclei (Azmitia and Segal, 1978).

Thus, at the neuroanatomical levels there is a close relationship between 5-HT and DA-containing neurons and this suggests that 5-HT could regulate the function of DA neurons via actions

on midbrain DA cell bodies and on DA terminals (see Chapter 12).

Serotonin/dopamine receptors

For detailed reviews of 5-HT receptor distribution and functional interactions with the dopaminergic system see Barnes and Sharp (1999) and Alex and Pehek (2007).

There are seven main classes of receptors for 5-HT, most of which exist as subtypes, resulting in at least 14 different receptors (Barnes and Sharp, 1999; Hoyer et al., 2002). The major 5-HT receptors which have been implicated in schizophrenia and in the action of antipsychotic drugs include the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT₆ receptors (Meltzer, 1999).

5-HT_{1A} receptors are present at high levels in the raphe nuclei where they act as autoreceptors to regulate raphe cell firing (Barnes and Sharp, 1999). These receptors are also present at high levels in limbic brain areas (such as hippocampus, lateral septum and cortical areas) which include cortico-limbic DA terminal brain areas (prefrontal cortex, amygdala and hippocampus). In contrast, levels of the 5-HT_{1A} receptor in basal ganglia are very low or not detectable. A recent electron microscopy study found co-labelling of the 5-HT_{1A} receptor with tyrosine hydroxylase in the VTA (Doherty and Pickel, 2001). In the prefrontal cortex, 5-HT_{1A} receptors are co-localised with 5-HT_{2A} receptors.

It has been hypothesised that a relatively high-affinity for the 5-HT_{2A} receptor compared to the D₂ receptor may explain the difference between 'typical' and 'atypical' antipsychotic drugs. Autoradiographic binding studies suggest that 5-HT_{2A} receptors appear to map onto the distribution of 5-HT axons from the dorsal raphe nuclei. For example, 5-HT innervation of the frontal cortex maps onto the laminar distribution of 5-HT_{2A} receptor-binding sites in this region. The 5-HT_{2A} receptor is mainly located on local (GABAergic) neurons, which may in turn regulate DA release, but is also present on cortical pyramidal projection neurons, which are glutamatergic (Burnet et al., 1995).

High levels of both 5-HT_{2C} receptor protein and mRNA have been found in several cortical areas,

in the hippocampus, striatum, septal nuclei, thalamic nuclei, brain stem nuclei and spinal cord (Alex and Pehek, 2007). Much work has focussed on the localisation and potential role of 5-HT_{2C} receptors in basal ganglia and its association with the dopaminergic system. In situ hybridisation studies have demonstrated that mRNA for the 5-HT_{2C} receptor is expressed in the VTA, subdivisions of the substantia nigra and in the terminal regions of the nigrostriatal and mesolimbic dopaminergic pathways, i.e. striatum and NAc.

The 5-HT₆ receptor (Monsma et al., 1993; Woolley et al., 2004) is predominantly expressed in the brain where, in the rat, it has been localised to the olfactory tubercle, NAc, striatum, hippocampus and cerebral cortex. Immunocytochemistry at the light and electron microscope levels has shown 5-HT₆ receptor-like immunoreactivity to be associated with dendritic processes in both the striatum and hippocampus of adult rats. This distribution is supported by autoradiographic studies, using the selective radioligand [¹²⁵I]SB-258585 (4-Iodo-*N*-[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-benzenesulfonamide), in both rats (Roberts et al., 2002) and human (East et al., 2002). Dual label immunohistochemistry (IHC) studies have indicated that 5-HT₆ receptors are localised on GABAergic neurons in the cerebral cortex, basal ganglia and hippocampus, implying that this receptor may modulate GABAergic neurotransmission in the brain.

DA receptors are encoded by five separate genes, D₁–D₅ (Civelli et al., 1993). For a detailed review of their structure and function see Missale et al. (1998). Two D₁-like receptor subtypes (D₁ and D₅) couple to the G protein G_s and activate adenylyl cyclase. The other receptor subtypes belong to the D₂-like subfamily (D₂, D₃, D₄) and are prototypic of G protein-coupled receptors that inhibit adenylyl cyclase and activate K⁺ channels. The D₂ and D₃ receptors vary in certain tissues and species as a result of alternative splicing, and the human D₄ receptor gene exhibits extensive polymorphic variation. The DA D₂ receptor is present in many areas of the central nervous system, but it is preferentially located in the SNc, the VTA, the striatum (which

includes the NAc shell and core and the dorsal striatum), the olfactory tubercle and the pituitary gland.

Serotonin/dopamine interactions in therapeutic agents for schizophrenia

The discovery of antipsychotic drugs was serendipitous and the subsequent theories of schizophrenia have largely capitalised on their mechanism of action (for reviews see Kapur and Mamo, 2003; Seeman, 2006). The first drugs used in the treatment of schizophrenia included reserpine, a DA and noradrenaline releaser, and chlorpromazine, which blocked DA receptors. Research focussed on developing new drugs which blocked the central DA D₂ receptors, but there were differences in the profile of newer antipsychotic drugs which were difficult to reconcile. This was exemplified by clozapine which was different from other antipsychotic drugs. Pre-clinically, clozapine was effective in some animal models of antipsychotic drug action (such as amphetamine and apomorphine climbing) but was inactive in others (such as amphetamine stereotypy, induction of catalepsy). Clinically, clozapine provided an antipsychotic effect without neuroleptic activity in that it did not produce marked sedation or motor retardation, and had a much reduced propensity to develop extrapyramidal side effects (EPS). This led to the term atypical antipsychotic and there has been much debate as to what accounts for atypical antipsychotic activity at the pharmacological and physiological level (Kapur and Mamo, 2003). This has been confounded by the fact that the antipsychotic drugs show a rich pharmacology in that they have effects at multiple receptors (Arnt and Skarsfeldt, 1998; Roth et al., 2004). Many of these interactions are associated with the numerous side-effects associated with antipsychotic drug use, e.g. orthostatic hypotension is believed to be associated with the alpha-adrenergic receptor (Drici and Priori, 2007), weight gain has been linked with an action at the histamine H₁ receptor (Kroeze et al., 2003; Garzya et al., 2007). Based on the different receptor interaction profile of typical and atypical antipsychotic drugs, the role of an

interaction with serotonergic receptors was proposed as a key feature of the efficacy of these drugs (Meltzer et al., 2003). However, the key mechanism responsible for driving the therapeutic efficacy of the typical and atypical antipsychotic drugs remains antagonism of the DA D₂ receptor. The most recent advance has been the development of aripiprazole which offers a novel mechanism, namely partial agonist activity at the DA D₂ receptor, for efficacy in schizophrenia with low side-effects such as EPS and prolactin elevation.

Dopamine D₂ receptors — antagonists and partial agonists

Although all currently used antipsychotic drugs have the same mechanism of action, namely to block DA D₂ receptors in the brain, it is clear that not all antipsychotic drugs have the same clinical profile and that some of the differences can be related to differences in DA D₂ receptor blockade. Thus, antipsychotic drugs are a heterogeneous group in terms of both effectiveness and of adverse events, even in respect to side-effects that are related to D₂ receptor blockade such as elevations in prolactin levels and development of EPS. A meta-analysis comparing atypical and typical antipsychotic drugs indicated that clozapine, risperidone and olanzapine demonstrated significantly higher efficacy than typical antipsychotic drugs, whereas sertindole, quetiapine, ziprasidone and remoxapride did not differ in terms of efficacy from typical antipsychotic drugs (Davis et al., 2003). Most of the newer atypical antipsychotic drugs show only a small or transient prolactin elevation compared to the sustained and large prolactin elevation associated with typical antipsychotic drugs. However, risperidone and amisulpride are associated with marked prolactin elevation, which may reflect their high-peripheral drug levels, and with substantial DA receptor blockade in the pituitary (Kapur et al., 2002). Clozapine also lacked the adverse EPS (dystonia, Parkinsonism, akathisia, tardive dyskinesia) seen with drugs such as chlorpromazine and haloperidol. Although most of the newer atypical antipsychotic drugs show a relatively low risk of inducing EPS, they differ in their impact on

neurological functioning. The atypical antipsychotic can be ranked by EPS risk in the following order: clozapine < quetiapine < olanzapine = ziprasidone < high dose risperidone (Tarsy et al., 2002).

When trying to develop new antipsychotic drugs, it is important to balance the efficacy afforded by DA receptor blockade with the side-effects mediated by D₂ receptor blockade. With the advent of neuroimaging, it has become apparent that it is critical to optimise the level of DA D₂/D₃ receptor blockade in the striatum (current ligands do not discriminate between these receptor types). Thus, positron emission tomography (PET) studies have suggested that the presence of a therapeutic window for striatal DA D₂/D₃ receptor occupancy, with 50–70% being optimal levels for antipsychotic response and occupancies greater than 80% being associated with increased incidence of EPS (Kapur and Mamo, 2003). This window can be achieved by a number of different mechanisms, including targeting low-affinity/fast off-rate. Clozapine and quetiapine both show low and/or transient occupancy of brain DA D₂ receptors and this may be explained by the observation that these two antipsychotic drugs rapidly dissociate from the D₂ receptor (Seeman, 2006). This may also explain their low propensity to induce elevations in prolactin levels and motoric side-effects and suggests that sustained and full blockade of the DA D₂ receptor is not required for a therapeutic effect. While most of the imaging studies have examined striatal DA D₂ receptor occupancy for technical reasons, some studies have shown high levels of DA D₂ receptor occupancy in the cortex (>90%) with antipsychotic drug treatment. Since striatal DA D₂ receptor occupancy is probably more related to the side-effects of D₂ receptor occupancy, whereas cortical DA D₂ receptor occupancy may be more related to the therapeutic efficacy, it was suggested that this limbic selectivity could be key in the mechanism of action of atypical antipsychotic drugs (Bigliani et al., 2000).

A recent novel alternative mechanism for balancing these effects is that of partial agonism at the DA D₂ receptor (Tamminga, 2002). Aripiprazole is a novel drug which has antipsychotic efficacy with a low risk of EPS and no prolactin elevation but which shows high (>90%) striatal D₂ receptor occupancy (Yokoi et al., 2002).

In vitro, aripiprazole displays agonist properties with a low-intrinsic activity at the DA D₂ receptor (Lawler et al., 1999). In vivo, aripiprazole has been shown to exhibit both antagonist (e.g. blockade of apomorphine-induced stereotypy) and agonist (e.g. reduction of increased DA synthesis in reserpine treated rats) properties (Kikuchi et al., 1995). Although aripiprazole does interact with other receptor subtypes, it was suggested that partial agonist activity at the DA D₂ receptor accounts for most of the novel clinical profile of aripiprazole with little or no contribution from an action at 5-HT receptors (Natesan et al., 2006; Wood and Reavill, 2007). In support of this, only relatively low 5-HT₂ and 5-HT_{1A} receptor occupancy is seen at clinically effective doses in humans (Mamo et al., 2007). It has been suggested that aripiprazole is a functionally selective agonist in that it has different profiles of activity on distinct signal pathways, e.g. cAMP compared to ERK (Mailman, 2007). How this relates to the in vivo and clinical profile of aripiprazole is difficult to predict, but it has been suggested that partial agonist activity at the DA D₂ receptor alone is sufficient to account for aripiprazole's pre-clinical profile (Wood and Reavill, 2007). However, it is clear that aripiprazole has marked a major change in antipsychotic drug development in that it provides a novel mechanism (other than 5-HT_{2A} receptor blockade), namely D₂ partial agonist activity, to achieve 'atypicality' as seen by a reduced EPS liability. Although initial data suggested that aripiprazole may not be as clinically effective as olanzapine and clozapine in controlled trials (Davis et al., 2003), this may not be the case in clinical practice due to an improved compliance and reduced side-effect liability seen with aripiprazole (Kerwin et al., 2007).

Dopamine/5-HT_{1A} receptor interaction

There are many lines of evidence to suggest that 5-HT_{1A} receptor activation may have beneficial effects on the antipsychotic properties of DA D₂ receptor blockade and this has been extensively covered elsewhere (Milan, 2000; Meltzer et al., 2003; Newman-Tancredi et al., 2007). Thus, 5-HT_{1A} receptor activation attenuates neuroleptic-induced catalepsy, suggesting a reduction in EPS liability.

5-HT_{1A} receptor agonists can stimulate the release of DA in the prefrontal cortex as well as potentate the increase in DA release seen with DA D₂ receptor blockade. Further, 5-HT_{1A} receptor activation may have beneficial effects in mood and mixed anxiety–depressive states. Most importantly, clinical trials using the 5-HT_{1A} receptor partial agonist drugs buspirone and tandospirone have shown an improvement in cognitive and negative symptoms (Goff et al., 1991; Sumiyoshi et al., 2001).

However, the level of 5-HT_{1A} receptor activation/blockade and the relative balance of 5-HT_{1A} and DA D₂ receptor activity (5-HT_{1A}:D₂ ratio) seems crucial to obtain the desired pharmacological and clinical profile. For example, pre-clinical studies in both rodents and primates suggest that it is predominantly antagonist activity at the 5-HT_{1A} receptor, which is required for alleviation of cognitive deficits (Harder and Ridley, 2000) whereas agonist activity at the 5-HT_{1A} receptor may impair cognitive function (Luttgen et al., 2005). It should be noted that although pre-clinical studies support a role for the 5-HT_{1A} receptor in cognition, the pro-cognitive signal seen in the tandospirone add-on study was small (Goff et al., 1991; Sumiyoshi et al., 2001). In contrast, it is predominantly agonist activity at the 5-HT_{1A} receptor which is required to attenuate antipsychotic drug-induced catalepsy in rats and compounds with low-intrinsic activity lack this property (Bardin et al., 2006). Thus, a narrow window of partial agonist activity is required to show cognitive enhancement and attenuate antipsychotic–drug-induced catalepsy. Further, it also appears that the balance of 5-HT_{1A}/D₂ receptor activity is important (Newman-Tancredi et al., 2007). For example, buspirone exhibits a pronounced preference for the 5-HT_{1A} receptor, lacks antipsychotic activity in the clinic, but can be used as an add-on to an antipsychotic drug therapy improving mood and anxiety scores and reducing EPS liability (Goff et al., 1991).

Dopamine/5-HT_{2A} and 5-HT_{2C} receptor interactions

The 5-HT_{2A} receptor has been proposed to play a critical role in the action of atypical antipsychotic

drugs (Meltzer, 1999; Meltzer et al., 2003). Indeed, it has been proposed that potent 5-HT_{2A} receptor antagonism together with weak DA D₂ receptor antagonism is the principal differentiating feature between atypical and typical antipsychotic drugs. There is also substantial evidence for a role of 5-HT_{2A} receptors in schizophrenia as well as in the action of antipsychotic drugs (Meltzer, 1999; Kapur and Mamo, 2003; Meltzer et al., 2003). For example, several post-mortem studies have found a decrease in cortical 5-HT_{2A} receptor binding and mRNA. There is considerable information showing that atypical antipsychotic drugs at clinically effective doses, show high levels of occupancy of central 5-HT_{2A} receptors, exceeding their occupancy of DA D₂ receptors in the striatum (Fard et al., 1995; Kapur et al., 1999). There is also much evidence showing that 5-HT_{2A} receptor antagonists modulate dopaminergic activity and, importantly they have been shown to facilitate antipsychotic drug-induced DA release in the prefrontal cortex (Meltzer, 1999; Meltzer et al., 2003). It has also been shown that 5-HT_{2A} receptor antagonists display activity in pre-clinical models of antipsychotic drug action and, in particular, are effective in animal models of negative symptoms (Schmidt et al., 1995).

Although there is considerable evidence to support this concept, it is clear that there are also anomalies. For example, high doses of the typical antipsychotic drug chlorpromazine can induce 5-HT_{2A} receptor blockade equivalent to that of clozapine (Trichard et al., 1998). Risperidone, which has one of the highest 5-HT_{2A} receptor affinities and shows very high 5-HT_{2A} receptor occupancy even at subclinical dose levels, is associated with the development of EPS in patients, albeit at high doses (Arnt and Skarsfeldt, 1998). Lastly, there is little clinical evidence that 5-HT_{2A} receptor antagonists are effective in schizophrenia or in ameliorating the negative symptoms of this disease. It has been suggested that some of these anomalies may reflect an involvement of the 5-HT_{2C} receptor (Wood et al., 2006). Thus, most atypical antipsychotic drugs have similar affinities for the 5-HT_{2A} and 5-HT_{2C} receptor subtypes. However, risperidone, which has a high-liability to induce EPS, has a

relatively low-affinity for the 5-HT_{2C} receptor compared to its affinity for the 5-HT_{2A} receptor. Using antagonists selective for the 5-HT_{2A} and 5-HT_{2C} receptor subtypes, it was shown that selective antagonism of the 5-HT_{2C} receptor was responsible for attenuating the cataleptic behaviour induced by DA receptor blockade (Wood et al., 2006). These pre-clinical studies suggest that it is the associated high-affinity of antipsychotic drugs for the 5-HT_{2C} receptor, which is limiting their EPS liability rather than their affinity for the 5-HT_{2A} receptor.

There is also evidence to suggest that as well as reducing EPS liability of antipsychotic drugs, antagonism of the 5-HT_{2C} receptor may have therapeutic benefits (Wood et al., 2001; Wood, 2005). Thus, 5-HT_{2C} receptor blockade increases DA release in the NAc (Berg et al., 2006) and, following repeated administration, preferentially decreases cell firing in dopaminergic neurons originating in the VTA and projecting to meso-limbic areas (Wood et al., 2001). 5-HT_{2C} receptor antagonism may also have beneficial effects on negative symptoms as there is substantial evidence of activity in pre-clinical models of anxiety and mood (Wood, 2003). Interestingly, recent evidence suggests that some of these properties are also associated with agonism at the 5-HT_{2C} receptor (Marquis et al., 2007). Accordingly, chronic treatment with the selective 5-HT_{2C} receptor agonist WAY-163909 was also associated with a selective reduction in the firing of DA neurons originating in the VTA. The observation that chronic treatment with compounds displaying agonist and antagonist properties at the 5-HT_{2C} receptor produces a similar response has been reported before, e.g. in the stress-induced anhedonia model (Moreau et al., 1996). It was proposed that this may reflect paradoxical receptor down-regulation seen after chronic treatment with both agonists and antagonists.

Dopamine/5-HT₆ receptor interactions

Interest in the 5-HT₆ receptor as a potential target for antipsychotic drugs started when it was observed that some antipsychotic drugs (notably clozapine) were potent antagonists of this receptor

(Monsma et al., 1993). The receptor is present at high levels in the hippocampus, NAc and striatum of rats (Gerard et al., 1997). Several studies have shown that 5-HT₆ receptor antagonists can improve memory and learning in a wide number of animal models (Rogers and Hagan, 2001; Woolley et al., 2004; Hirst et al., 2006) including: models of working memory (novel object recognition) and spatial memory (water maze); models of learning deficits (aged rats) and in those impaired with cholinergic antagonists (passive avoidance and a model of recognition memory). Furthermore, it has been shown that 5-HT₆ receptor antagonists can increase DA release in the medial prefrontal cortex, potentiate amphetamine-induced DA release in the striatum (Lacroix et al., 2004) and, importantly, they can potentiate antipsychotic drug-induced DA efflux in the medial prefrontal cortex or hippocampus (Li et al., 2007). This has raised the possibility that 5-HT₆ receptor antagonism may improve the efficacy against positive symptoms when added to an antipsychotic drug and that they may improve the cognitive deficits associated with schizophrenia.

Serotonin/dopamine interactions in therapeutic agents for depression and anxiety

The pathophysiology of depression has been dominated by the monoamine hypothesis of depression, ever since the serendipitous discovery 50 years ago of the clinical efficacy of MAO inhibitors (MAOI; e.g. iproniazid) and tricyclic antidepressants (TCAs; e.g. imipramine). This hypothesis was based on deficits in 5-HT and noradrenaline neurotransmission (Maes and Meltzer, 2000; Schatzberg and Schildkraut, 2000) and, at a later date, was extended to include abnormalities in dopaminergic neurotransmission (Willner, 2000) and the specific role of the mesolimbic and mesocortical DA pathways in depression (Nestler and Carlezon, 2006).

The mesocortical dopaminergic pathway arises from the VTA and projects to the frontal and temporal cortices, particularly the anterior cingulate, entorhinal and prefrontal cortices.

This pathway is believed to be important for concentration and executive functions such as working memory. The mesolimbic pathway also arises in the VTA but projects to the ventral striatum (including the NAc), bed nucleus of the stria terminalis, hippocampus, amygdala and septum. It is particularly important for motivation, the experience of pleasure and reward (Dunlop and Nemeroff, 2007). These symptoms, experienced by depressed patients, are poorly treated by current antidepressant drugs that predominantly affect serotonergic and/or noradrenergic mechanisms (Slattery et al., 2004).

However, with the increased exploration of a variety of monoaminergic drugs pre-clinically and clinically, alone and/or in combination, coupled with an increase in the pharmacological understanding of brain neurocircuitry, an increased understanding in the significance of 5-HT and DA interactions are leading to a more rational drug design for psychiatric disorders such as major depressive disorder (MDD). MAO enzymes and transporters have inherent neurotransmitter substrate flexibility and, therefore, a number of targets are being investigated that could further help to explain the connectivity between these monoaminergic neuronal circuits and those that could offer more effective and safer treatment options for the future.

Serotonin/dopamine interactions and monoamine oxidase (MAO) enzymes

MAO enzymes catalyse the major inactivation pathway for the catecholamine neurotransmitters, noradrenaline, adrenaline, DA and also 5-hydroxytryptamine. They exist as two isoforms, MAO-A and -B, and are flavoenzymes located in the outer membrane of mitochondria that oxidatively deaminate these neurotransmitters. Although both isoforms can deaminate DA, MAO-A preferentially oxidises 5-HT and noradrenaline and is inhibited by low concentrations of clorgyline. MAO-B has higher affinity for phenylethylamine and benzylamine, and is inhibited by low concentrations of deprenyl. Differences in the distribution of the two isoforms across species are reported, but in general MAO-A is the predominant isoform in

peripheral tissues, and MAO-B is mainly found in the CNS.

Irreversible, non-selective MAO inhibitors have been utilised for many years in psychiatric and neurological disorders (Youdim and Bakhle, 2006) and have comparable efficacy to TCAs in different forms of depression. However, they have not been widely used in the clinic due to a range of major side-effects including hepatotoxicity, orthostatic hypotension, hypertensive crisis and the need for dietary restrictions due to the tyramine 'cheese effect' (Yamada and Yasuhara, 2004). Consequently, safer MAO inhibitor alternatives with a selective and/or reversible mode of action were developed. However, selective agents such as the MAO-B inhibitor, selegiline, still retained side-effect issues including anorexia/nausea, dry mouth, dyskinesia, orthostatic hypotension, musculoskeletal injuries and cardiac arrhythmias. Further, selegiline use is still associated with the need for dietary restrictions (Patkar et al., 2007). The initial reversible MAO-A inhibitors (RIMAs), moclobemide and brofaromine, have been found to be as effective as selective serotonin re-uptake inhibitors (SSRIs) with an acceptable side-effect profile (Lotufo-Neto et al., 1999). These drugs represent a useful addition to the therapeutic arsenal particularly for patients who cannot tolerate the older MAOIs or who do not respond to SSRIs. The development of RIMAs with improved pharmacodynamic and pharmacokinetic properties (such as once daily dosing) is still being pursued.

Serotonin/dopamine interactions and monoamine transporters

The control of biogenic amine neurotransmission at the nerve terminal is predominantly mediated by monoamine transporters. Their primary function is to allow the rapid synaptic clearance of neurotransmitter back into the nerve terminal. This energy-dependent process is driven by an electrochemical gradient, which is created and maintained by the plasma membrane associated Na^+/K^+ ATPases (Rudnick and Clark, 1993). 5-HT, noradrenaline and DA transporters (SERT, NET and DAT respectively) are large 12 transmembrane proteins that are distinguishable by

their localisation, structure, stoichiometry, substrate preference and pharmacology (Owens et al., 1997; Hoffman et al., 1998; Sanchez and Hyttel, 1999; Iversen, 2006; Torres and Amara, 2007). For example, the uptake of DA is accompanied by two transported Na^+ ions, while 5-HT and noradrenaline are co-transported with only a single Na^+ ion (Gu et al., 1994). Although these transporters have a pharmacological preference for uptake of their own cognate neurotransmitter, they may also transport each others main substrates, albeit with lower affinity (Amara and Kuhar, 1993). Therefore, cross-talk between the fundamental re-uptake systems exist, which is also evident by the ability of neurotoxins such as MDMA, fenfluramine and MPTP, to be taken up by these transporter-mediated processes (Sprague et al., 1998). Many psychostimulant drugs such as amphetamine and cocaine have a primary pharmacological action — mediated by the monoamine transporters. This body of evidence has led to the monoamine transporters becoming important drug targets for treating psychiatric disorders such as addiction, compulsive behaviour, depression, anxiety and attention deficit hyperactivity disorder (ADHD) by regulating monoamine availability (Jayanthi and Ramamoorthy, 2005; Surratt et al., 2005; White et al., 2005; Gether et al., 2006).

The most widely studied monoamine transporter in psychiatric research has been SERT with the rationale design of SSRIs in the 1980s. The SSRIs remain the most commonly used medication for the treatment of depression and associated disorders. However, they are only effective in ~70% of patients (Nierenberg and DeCecco, 2001), with ~40% of responders still displaying a number of residual symptoms (Fava, 2006). Further, despite maintenance of drug treatment there is also a high-frequency of relapse (~15–40%; Geddes et al., 2003; Fava, 2006; Rush et al., 2006). Additionally the tolerability of SSRIs, although generally better than TCAs, can limit compliance and restrict dose ranging due to a number of serious side-effects which occur in 10–20% of patients and which include sexual dysfunction, insomnia and nausea (Mace and Taylor, 2000). Abrupt discontinuation of some of the SSRIs may result in a withdrawal syndrome that includes dizziness, anxiety,

irritability and flu-like symptoms. However, the SSRIs have proved a major advance in the treatment of a wide range of anxiety and depressive disorders including dysthymia, generalised anxiety disorder, obsessive-compulsive disorder (OCD), panic disorder, phobic disorder, post-traumatic stress disorder, premenstrual dysphoric disorder and bulimia. However, SSRIs, like TCAs, have a slow onset of action with antidepressant activity often observed only after 2–8 weeks of continuous treatment. The development of [^{11}C] DASB as a PET ligand has allowed functional imaging to that >80% SERT occupancy is needed for clinical efficacy (Meyer et al., 2004).

Recent advances in molecular pharmacology and in receptor modelling have suggested alternative mechanisms for the rational design of drugs that selectively inhibit SERT. Molecular pharmacological studies have suggested the existence of an allosteric site on SERT and, further, that escitalopram can be considered as an allosteric 5-HT re-uptake inhibitor. Escitalopram was found to have an action on two sites (the primary site and the allosteric site) on the 5-HT transporter and this may be responsible for the longer binding to, and therefore greater inhibition of, the 5-HT transporter (Sanchez, 2006). Such a dual action may also explain why escitalopram reaches higher SERT occupancies and has superior clinical efficacy than the racemate parent (Lundberg et al., 2007). Indeed, allosteric modulators of SERT have been proposed (Boos et al., 2006) and advances in molecular biology have started to identify specific antidepressant sensitive interacting binding sites on the 5-HT transporter (Henry et al., 2006). The recent identification of the bacterial leucine transporter as a homologue of the monoamine transporter family has enabled a model of the transporter to be developed. This has been used to identify the recognition sites for desipramine, which are responsible for blocking substrate uptake (Zhou et al., 2007). With the recent developments of heterozygous knockouts (Kalueff et al., 2007) and gene silencing technologies (Hoyer, 2007), further insight into the functioning and regulation of SERT are likely to be gained.

The increased understanding of the close interaction between the monoamine networks has led

to the development of selective compounds which inhibit more than one transporter at the same time. This approach has led to the development of duloxetine and venlafaxine, which are selective 5-HT and noradrenaline re-uptake inhibitors (SNRIs). However, there is no consensus that combining these effects offers a significant advantage over SSRIs. Recent meta-analysis of a large number of comparative trials suggests, at best, a modest efficacy advantage of the SNRI over the SSRI class (Papakostas et al., 2007a). Bupropion (Foley et al., 2006), is a selective DA and noradrenaline re-uptake inhibitor which is effective in MDD and seasonal affective disorder. Its major advantage over the SSRIs is a reduction in sexual dysfunction side-effects with no improvement in efficacy or onset.

There still remains a great need for faster acting, safer and more effective treatments for depression. To this end, a number of triple re-uptake inhibitors are now being developed, including DOV 216,303 (from DOV, Phase II), DOV 21,947 (Phase I), NS-2359 (Phase II, Neurosearch and GlaxoSmith Kline) and SEP-225289 (Phase I, Sepracor). By simultaneously inhibiting the re-uptake of all three monoamines there is an expectation that triple re-uptake inhibitors will offer significant advantages over both single and dual re-uptake inhibitors. For example, dopaminergic receptor agonists (bromocriptine, pergolide or pramipexole) and DA elevating agents (methylphenidate and modafinil) have been successfully used in resolving some of the symptoms not fully treated by existing antidepressant treatments such as fatigue, somnolence, apathy as well as cognitive and executive dysfunction (Fava, 2006). Additionally, bupropion is effective in managing SSRI residual symptoms and augments the therapeutic response to citalopram, producing a reduction in both the number and severity of symptoms as well as side-effects and adverse events (Trivedi et al., 2006). The addition of DAT blockade into an SNRI-like profile is therefore predicted to improve symptoms of mood, such as anhedonia, melancholia, lack of motivation, as well as cognitive symptoms that are observed in many depressed patients and, further, may counteract the sexually-related side-effects associated with SERT blockade.

A potential major issue in the development of DAT and triple re-uptake inhibitors is that of abuse liability (as seen with cocaine) that may arise due to inhibition of DAT. In this respect, PET imaging studies have demonstrated a risk of abuse potential associated with >50% DAT occupancy or fast association binding such as that seen with cocaine. Notably, however, low potency compounds, such as bupropion, only need to achieve 30% DAT occupancy for their antidepressant effect, where the binding association kinetic would be slow. Indeed bupropion has not been shown to demonstrate any reinforcing properties (Volkow et al., 2005). A slow and long lasting DAT blockade, such as that observed with radafaxine in PET studies (Volkow et al., 2005), may indeed avoid the potential for abuse liability. DOV and the Mayo foundation have suggested that triple re-uptake inhibitors, which display a reduced activity at DAT compared to that at SERT and NET, may possess a reduced potential for abuse liability (Shaw et al., 2007). The pharmacological profiles of triple re-uptake inhibitors currently in the clinic or in development, however show a more balanced monoamine transporter binding profile (Chen and Skolnick, 2007), suggesting that pre-clinical and clinical studies may be necessary to discharge the abuse potential liability if their DAT binding kinetic profile is no different from cocaine.

Serotonin/dopamine interactions: 5-HT receptors mediating dopaminergic function

5-HT₂ receptors and the dopaminergic system

Disinhibition of the mesolimbic DA system may underlie the mechanism of action of several antidepressant drugs (Cervo and Samanin, 1987, 1988). Acute administration of SSRIs has been shown to inhibit VTA firing with no effect on that in the SN and, further, the effect on VTA cell firing is not apparent after chronic SSRI treatment. Additionally chronic treatment with a variety of SSRIs induces tolerance to the hypolocomotor effects of the 5-HT_{2C} receptor agonist, mCPP. Chronic treatment with many antidepressant drugs

leads to a down-regulation of the 5-HT₂ receptors, in both animals and humans (which may lead to increased dopaminergic firing), and has been proposed to be important for their antidepressant effect (Landén and Thase, 2006). Therefore, both 5-HT_{2C} receptor agonists and antagonists have been considered as potential therapeutic agents in regulating a hypofunctional DA system associated with hypersensitive inhibitory 5-HT₂ receptors located on dopaminergic neurons. Behavioural and neurochemical data have additionally suggested that 5-HT₂ receptor antagonists may augment antidepressant activity (Cremers et al., 2004; Marek et al., 2005; Pierre Olié and Kasper, 2007). More recent studies have suggested that this effect of 5-HT_{2C} receptor antagonism may be mediated by GABAergic mechanisms in the hippocampus, highlighting that the connectivity of 5-HT and dopaminergic interactions are indeed highly complex and not yet fully understood (Cremers et al., 2007). Since 5-HT_{2C} receptors have been found to be expressed on GABAergic neurons (but not on 5-HT-containing cells) of the anterior raphe nuclei (Serrats et al., 2005), it is conceivable that blockade of these receptors could disinhibit serotonergic neurons leading to an increased 5-HT tone to the VTA, which, in turn, can excite 5-HT_{2A} receptors to depolarise DA neurons. The addition of low doses of atypical antipsychotic drugs, which saturate 5-HT_{2A} receptors, enhanced the therapeutic effect of SSRIs in patients with major depression as well as treatment-refractory OCD. As such, antipsychotic drug add-on to existing antidepressant therapy is increasingly being used in the clinic (Carvalho et al., 2007; Papakostas et al., 2007b; Shelton, 2007; Skapinakis et al., 2007). With the suggestion that depression may be associated with positive and negative symptoms in acute schizophrenia (Muller, 2007), antidepressant augmentation of antipsychotic drug treatment in schizophrenia is also being explored. Future drugs combining key features of antidepressant and atypical antipsychotic agents could offer new promise for patients suffering from OCD, post-traumatic stress disorder, panic disorder, generalised anxiety disorder and depression (Rasmussen, 2006). By incorporating 5-HT₂ receptor antagonism with current

antidepressant treatment, an increased efficacy may be expected together with a reduction in the side-effect potential such as sleep disturbances and centrally mediated sexual dysfunction. Although this strategy has been employed, e.g. amitriptyline, clompiramine, these antidepressant drugs display significant side-effects mediated by additional pharmacologies. Nefazodone was a relatively new antidepressant drug that has combined 5-HT and NA re-uptake inhibition with 5-HT_{2A} receptor antagonism (Schechter et al., 1999). In the clinic, nefazodone is an effective antidepressant and is claimed to have a low propensity for side-effects such as weight gain or sexual dysfunction, but was withdrawn due to liver toxicity problems (Desanty and Amabile, 2007). To explore further the influence of dopaminergic and serotonergic interactions in specific depressed patient populations, molecules with the appropriately balanced poly-pharmacology are required. In this respect, the antipsychotic drug quetiapine (Seroquel) is being investigated as a monotherapy in MDD Phase III trials as it has potent 5-HT_{2A} receptor antagonism and agonist activity at the 5-HT_{1A} receptor (McIntyre et al., 2007). Furthermore, aripiprazole (Abilify) has recently been approved as an add-on therapy for treatment-resistant depressed patients.

5-HT_{1A} receptors and the dopaminergic system

As discussed previously, the atypical antipsychotic drug quetiapine is being investigated as a novel treatment for depression. Quetiapine itself has a rich pharmacology (including affinity for the DA D₂ and D₃ receptor, 5-HT_{1A} and 5-HT_{2A} receptors and α_2 -adrenoceptor) and there is emerging evidence that the principal human plasma metabolite of quetiapine, *N*-desalkyl quetiapine, may also contribute to the overall clinical profile. Indeed, this metabolite has high-affinity for, and is a potent inhibitor of, the noradrenergic transporter, and displays partial agonist activity at the 5-HT_{1A} receptor. 5-HT_{1A} receptor activation results in an increase in prefrontal cortex dopaminergic neurotransmission and in local DA release in NAc (Ichikawa and Meltzer, 1999). In a genetically selected rat model of depression a

correlation between depressive behaviour and the absence of DA responsiveness to 5-HT stimulation in NAc was apparent and, furthermore, chronic antidepressant drug treatment normalised both the 5-HT–DA interaction and the depressive behaviour (Zangen et al., 2001).

The 5-HT_{1A} receptor has been proposed to be important in the mechanism of action of antidepressant drugs, thus the delayed onset of effect of current antidepressant drug treatment has been linked to the down-regulation of the 5-HT_{1A} autoreceptor in the midbrain raphe (Blier and Ward, 2003). Combination strategies with 5-HT_{1A} receptor agonists have been shown to accelerate this down-regulation and, therefore, may reduce the delay to therapeutic onset (Artigas et al., 2006). Consequently, many 5-HT_{1A} receptor agonists have progressed into stand-alone and combination clinical studies (Osemozotan or MN-305 or MKC-242, Abe et al., 1996; PRX-00023, Gepirone, Leslie, 2001; de Paulis, 2007a, b). Indeed vilazodone, a 5-HT_{1A} receptor agonist and 5-HT re-uptake inhibitor has recently demonstrated clinical efficacy in Phase III (de Paulis, 2007). It therefore remains plausible that a differentiating drug can be expected from a 5-HT_{1A} receptor-based approach that can also affect, albeit indirectly, the dopaminergic system.

Summary and future directions

Psychiatric diseases are complex disorders with multiple symptoms which vary in their degree and expression. They appear to involve multiple neurotransmitters and pathways and are treated by drugs which are often described as having a rich pharmacology.

For schizophrenia, it is clear that there are multiple symptoms and these symptoms are differentially treated by current drugs. These symptoms include positive (delusions, hallucinations), negative (alogia, social withdrawal, flattened affect) and cognitive (working and verbal memory) domains. Antipsychotic drugs treat mainly the positive disorders with little effect on cognition and functional outcomes (CATIE trial, McEvoy et al., 2006) and, further, are ineffective in

many patients. While there is some evidence that clozapine may be more effective in treatment-resistant patients, the atypical antipsychotic agents do not show any improvement in effectiveness above that seen with typical antipsychotic agents (CATIE trial). Although there is some evidence to suggest that amisulpride may show modest efficacy in treating negative symptoms (Murphy et al., 2006), it still remains a fact that the current drugs do not treat cognitive and negative symptoms effectively. Recently, there has been a trend towards improving the quality of life of schizophrenics by developing treatments, which may improve psychosocial functioning using animal models of cognitive deficits and negative symptoms. Such approaches are illustrated by F15063, a compound with D₂/D₃ receptor antagonist, 5-HT_{1A} receptor agonist and D₄ receptor partial agonist properties, which partially alleviated phencyclidine-induced deficits in social interaction (a model of negative symptoms) and reversed cognitive deficits induced by either phencyclidine or scopolamine (Depoortere et al., 2007). Another approach has been that of incorporating antagonist activity at the 5-HT₆ receptor to provide cognitive benefits together with activity at the DA D₂ and D₃ receptors and at the 5-HT_{2A} and 5-HT_{2C} receptors to treat positive and mood symptoms (Garzya et al., 2007). However, as has been noted elsewhere (Lawrence, 2007) this is a challenging balancing act. It is important to get the right balance in the relative level of affinity and efficacy (for DA D₂ receptor and 5-HT_{1A} receptor partial agonism) so that the compound delivers the desired efficacy profile. It is also just as important to minimise undesirable activities which may lead to unwanted side effects including, e.g. weight gain associated with histamine H₁ receptor antagonism (Kim et al., 2007) and orthostatic hypotension associated with alpha-adrenoceptor blockade.

In a review of the future of drug development in schizophrenia, it was predicted that the future will likely start with the continued use of polypharmacy and augmentation strategies aimed at treating the multiple symptom domains of schizophrenia. This may be followed by the development of single compounds that can target

multiple domains at once while simultaneously decreasing side-effects, eliminating potential pharmacokinetic interactions and improving medication compliance (Gray and Roth, 2007). This review shows although this is indeed already happening, it remains a major challenge for the pharmaceutical industry.

Although it is evident that 5-HT and dopaminergic interactions have a profound influence on depressive disorders, there remains a major unmet clinical need, in terms of both efficacy and side-effects. This has stimulated researchers to identify additional putative differentiating drug targets (Rosenzweig-Lipson et al., 2007) that extend beyond the monoamine hypothesis (Hindmarch, 2002; Berton and Nestler, 2006). These include the clinical investigation of glucocorticoid receptor antagonists (Nihalani and Schwartz, 2007), CRF-1 receptor antagonists (Kehne, 2007) and tachykinin receptor antagonists (Huang and Williams, 2007). The glucocorticoid and CRF approaches target restoration of a dysfunctional hypothalamic-pituitary axis stress response observed in many depressed patients, and offers the potential to target a symptom-specific subset of depressed patients. The investigation of tachykinin receptor antagonists to regulate central substance P mediated fear and anxiety circuits offers a novel approach with the potential for delivering a differentiated clinical and side-effect profile (Alvaro and Di Fabio, 2007). It should be noted that such mechanisms may still influence monoaminergic systems indirectly, e.g. NK₁ receptors have been shown to control 5-HT-mediated neurotransmission via an influence on the raphe nucleus (Guiard et al., 2007) and that co-administration of a NK₁ receptor antagonist with an antidepressant drug such as a SSRI may have the therapeutic potential to improve the treatment of major depressive episodes in humans beyond that afforded by a SSRI alone (Chenu et al., 2007; Huang and Williams, 2007). Additionally, blockade of tachykinin NK₁ receptors attenuates stress-induced increases in extra-cellular noradrenaline and DA in the rat and gerbil medial prefrontal cortex (Renoldi and Invernizzi, 2006), again suggesting integrated circuitry within monoaminergic pathways.

Additional mechanisms that involve regulating intracellular signalling pathways, the glutamatergic system, cytokines and neurotrophic factors are also under further biological and chemical investigation within the pharmaceutical industry. Indeed, it is very likely that just as the serotonergic and dopaminergic systems interact, more novel biological substrates that potentially mediate disease and antidepressant response, also interlink with monoaminergic pathways. For example, pro-inflammatory cytokines that are elevated in some depressives have been suggested to regulate monoamine transporter function *in vitro* (Zhu et al., 2005). Additionally, new approaches are moving from regulating monoaminergic pathways at the neurotransmitter receptor level to that at the signalling level. For example, tamoxifen has recently been shown to be effective in acute mania, an action thought to be mediated by inhibition of protein kinase C (Zarate et al., 2007).

In summary, it is known that there is a close anatomical and functional interplay between the 5-HT and DA nervous systems. Deficits in such interactions and neuronal circuits are a major factor in many psychiatric disorders. In order to restore the balance in these neuronal circuits, new drugs will need to act on multiple targets to modulate their activity and treat multiple components and symptoms of the disease to improve efficacy. In the longer term, newer drugs will target the functional interplay between the 5-HT and DA systems at the cellular and pathway level and may treat disease-specific symptoms. The advancement of novel agents with novel pharmacological profiles and novel mechanisms of action into the clinic and the potential to treat symptom domains, e.g. cognition, is eagerly anticipated.

References

- Abe, M., Tabata, R., Saito, K., Matsuda, T., Baba, A. and Egawa, M. (1996) Novel benzodioxan derivative, 5-[3-(((2S)-1,4-benzodioxan-2-ylmethyl) amino)propoxy]-1,3-benzodioxole HCl (MKC-242), with anxiolytic-like and antidepressant-like effects in animal models. *J. Pharmacol. Exp. Ther.*, 278: 898–905.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113: 296–320.
- Alvaro, G. and Di Fabio, R. (2007) Neurokinin 1 receptor antagonists: current prospects. *Curr. Opin. Drug Discov. Dev.*, 10: 613–621.
- Amara, S.G. and Kuhar, M.J. (1993) Neurotransmitter transporters: recent progress. *Annu. Rev. Neurosci.*, 16: 73–93.
- Arnt, J. and Skarsfeldt, T. (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology*, 18: 63–101.
- Artigas, F., Adell, A. and Celada, P. (2006) Pindolol augmentation of antidepressant response. *Curr. Drug Targets*, 7: 139–147.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179: 641–668.
- Bardin, L., Kleven, M.S., Barret-Grevoz, C., Depoortere, R. and Newman-Tancredi, A. (2006) Antipsychotic-like vs. cataleptogenic actions in mice of novel antipsychotics having D-2 antagonist and 5-HT_{1A} agonist properties. *Neuropsychopharmacology*, 31: 1869–1879.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38: 1083–1152.
- Bell, D. (1965) Comparison of amphetamine psychosis and schizophrenia. *Am. J. Psychiatry*, 111: 701–707.
- Berg, K.A., Navailles, S., Sanchez, T.A., Silva, Y.M., Wood, M.D., Spampinato, U. and Clarke, W.P. (2006) Differential effects of 5-Methyl-1-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]carbamoyl]-6-trifluoromethylindone (SB 243213) on 5-hydroxytryptamine_{2C} receptor-mediated responses. *J. Pharmacol. Exp. Ther.*, 319: 260–268.
- Berton, O. and Nestler, E.J. (2006) New approaches to antidepressant drug discovery: beyond monoamines. *Nat. Rev. Neurosci.*, 7: 137–151.
- Bigliani, V., Mulligan, R.S., Acton, P.D., Ohlsen, R.I., Pike, V.W., Ell, P.J., Gacinovic, S., Kerwin, R.W. and Pilowsky, L.S. (2000) Striatal and temporal cortical D₂/D₃ receptor occupancy by olanzapine and sertindole *in vivo*: a [¹²³I]epidepride single photon emission tomography (SPET) study. *Psychopharmacology*, 150: 132–140.
- Blier, P. and Ward, N.M. (2003) Is there a role for 5-HT_{1A} agonists in the treatment of depression? *Biol. Psychiatry*, 53: 193–203.
- Boos, T.L., Greiner, E., Calhoun, W.J., Prisinzano, T.E., Nightingale, B., Dersch, C.M., Rothman, R.B., Jacobson, A.E. and Rice, K.C. (2006) Structure-activity relationships of substituted *N*-benzyl piperidines in the GBR series: synthesis of 4-(2-(bis(4-fluorophenyl)methoxy)ethyl)-1-(2-trifluoromethylbenzyl) piperidine, an allosteric modulator of the serotonin transporter. *Bioorg. Med. Chem.*, 14: 3967–3973.
- Bunney, W.E. and Davis, J.W. (1965) Norepinephrine in depressive reactions: a review. *Arch. Gen. Psychiatry*, 13: 483–494.
- Burnet, P.W.J., Eastwood, S.L., Lacey, K. and Harrison, P.J. (1995) The distribution of 5-HT_{1A} and 5-HT_{2A} mRNA in human brain. *Brain Res.*, 676: 157–168.
- Carvalho, A.F., Cavalcante, J.L., Castelo, M.S. and Lima, M.C. (2007) Augmentation strategies for treatment-resistant

- depression: a literature review. *J. Clin. Pharm. Ther.*, 32: 415–428.
- Cervo, L. and Samanin, R. (1987) Evidence that dopamine mechanisms in the nucleus accumbens are selectively involved in the effect of desipramine in the forced swimming test. *Neuropharmacology*, 26: 1469–1472.
- Cervo, L. and Samanin, R. (1988) Repeated treatment with imipramine and amitriptyline reduced the immobility of rats in the swimming test by enhancing dopamine mechanisms in the nucleus accumbens. *J. Pharm. Pharmacol.*, 40: 155–156.
- Chen, Z. and Skolnick, P. (2007) Triple uptake inhibitors: therapeutic potential in depression and beyond. *Expert Opin. Investig. Drugs*, 16: 1365–1377.
- Chenu, F., Guiard, B.P., Bourin, M. and Gardier, A.M. (2007) Antidepressant-like activity of selective serotonin reuptake inhibitors combined with a NK1 receptor antagonist in the mouse forced swimming test. *Behav. Brain Res.*, 172: 256–263.
- Civelli, O., Bunzow, G.R. and Grandy, D.K. (1993) Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.*, 33: 281–307.
- Cremers, T.I., Giorgetti, M., Bosker, F.J., Hogg, S., Arnt, J., Mork, A., Honig, G., Bogeso, K.P., Westerink, B.H., den Boer, H., Wikstrom, H.V. and Tecott, L.H. (2004) Inactivation of 5-HT_{2C} receptors potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology*, 29: 1782–1789.
- Cremers, T.I., Rea, K., Bosker, F.J., Wikstrom, H.V., Hogg, S., Mork, A. and Westerink, B.H. (2007) Augmentation of SSRI effects on serotonin by 5-HT_{2C} antagonists: mechanistic studies. *Neuropsychopharmacology*, 32: 1550–1557.
- Davis, J.M., Chen, N. and Glick, I.D. (2003) A meta-analysis of the efficacy of second-generation antipsychotics. *Arch. Gen. Psychiatry*, 60: 553–564.
- de Paulis, T. (2007a) Drug evaluation: PRX-00023, a selective 5-HT_{1A} receptor agonist for depression. *Curr. Opin. Investig. Drugs*, 8: 78–86.
- de Paulis, T. (2007b) Drug evaluation: Vilazodone — a combined SSRI and 5-HT_{1A} partial agonist for the treatment of depression. *Drugs*, 10: 193–201.
- Depoortere, R., Auclair, A.L., Bardin, L., Bruins Slot, L., Kleven, M.S., Colpaert, F., Vacher, B. and Newman-Tancredi, A. (2007) F15063, compound with D₂/D₃ antagonist, 5-HT_{1A} agonist and D₄ partial agonist properties: (III) activity in animal models of cognition and negative symptoms. *Br. J. Pharmacol.*, 151: 266–277.
- DeSanty, K.P. and Amabile, C.M. (2007) Antidepressant-induced liver injury. *Ann. Pharmacother.*, 41: 1201–1211.
- Doherty, M.D. and Pickel, V.M. (2001) Targeting of serotonin 1A receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain. *J. Comp. Neurol.*, 433: 390–400.
- Drici, M.-D. and Priori, S. (2007) Cardiovascular risks of atypical antipsychotic drug treatment. *Pharmacoevidenciol. Drug Saf.*, 16: 882–890.
- Dunlop, B.W. and Nemeroff, C.B. (2007) The role of dopamine in the pathophysiology of depression. *Arch. Gen. Psychiatry*, 64: 327–337.
- East, S.Z., Burnet, P.W.J., Leslie, R.A., Roberts, J.C. and Harrison, P.J. (2002) 5-HT₆ receptor binding sites in schizophrenia and following antipsychotic drug administration: autoradiographic studies with [¹²⁵I]SB-258585. *Synapse*, 45: 191–199.
- Fard, L., Nyberg, S., Oxenstierna, G., Nakashima, Y., Halldin, C. and Ericsson, B. (1995) Positron emission tomography studies on D₂ and 5-HT₂ receptor binding in risperidone-treated schizophrenic patients. *J. Clin. Psychopharmacol.*, 15: 19S–23S.
- Fava, M. (2006) Pharmacological approaches to the treatment of residual symptoms. *J. Psychopharmacol.*, 20: 29–34.
- Foley, K.F., DeSanty, K.P. and Kast, R.E. (2006) Bupropion: pharmacology and therapeutic applications. *Expert Rev. Neurother.*, 6: 1249–1265.
- Garzya, V., Forbes, I.T., Gribble, A.D., Hadley, M.S., Lightfoot, A.P., Payne, A.H., Smith, A.B., Douglas, S.E., Cooper, D.G., Stansfield, I.G., Meeson, M., Dodds, E.E., Jones, D.N.C., Wood, M., Reavill, C., Scorer, C.A., Worby, A., Riley, G., Eddershaw, P., Ioannou, C., Donati, D., JimHagan, J.J. and Ratti, E.A. (2007) Studies towards the identification of a new generation of atypical antipsychotic agents. *Bioorg. Med. Chem. Lett.*, 17: 400–405.
- Geddes, J.R., Carney, S.M., Davies, C., Furukawa, T.A., Kupfer, D.J., Frank, E. and Goodwin, G.M. (2003) Relapse prevention with antidepressant drug treatment in depressive disorders: a systematic review. *Lancet*, 361: 653–661.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Research*, 746(1–2): 207–219.
- Gether, U., Andersen, P.H., Larsson, O.M. and Schousboe, A. (2006) Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol. Sci.*, 27: 375–383.
- Geyer, M.A. (1998) Behavioural studies of hallucinogenic drugs in animals: implications for schizophrenia research. *Pharmacopsychiatry*, 31(Suppl 2): 73–79.
- Goff, D.C., Midha, K.K., Brotman, A.W., McCormick, S., Waites, M. and Amico, E.T. (1991) An open trial of buspirone added to neuroleptics in schizophrenic patients. *J. Clin. Psychopharmacol.*, 11: 193–197.
- Gray, J.A. and Roth, B.L. (2007) The pipeline and future of drug development in schizophrenia. *Mol. Pharmacol.*, 12: 904–922.
- Gu, H., Wall, S.C. and Rudnick, G. (1994) Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J. Biol. Chem.*, 269: 7124–7130.
- Guiard, B.P., Guilloux, J.P., Reperant, C., Hunt, S.P., Toth, M. and Gardier, A.M. (2007) Substance P neurokinin 1 receptor activation within the dorsal raphe nucleus controls serotonin release in the mouse frontal cortex. *Mol. Pharmacol.*, 72: 1411–1418.
- Harder, J.A. and Ridley, R.M. (2000) The 5-HT_{1A} antagonist, WAY 100 635, alleviates cognitive impairments induced

- by dizocilpine (MK-801) in monkeys. *Neuropharmacology*, 3: 547–552.
- Henry, L.K., Field, J.R., Adkins, E.M., Parnas, M.L., Vaughan, R.A., Zou, M.F., Newman, A.H. and Blakely, R.D. (2006) Tyr-95 and Ile-172 in transmembrane segments 1 and 3 of human serotonin transporters interact to establish high affinity recognition of antidepressants. *J. Biol. Chem.*, 281: 2012–2023.
- Herve, D., Pickel, V.M., Tong, H.J. and Beaudet, A. (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. *Brain Res.*, 435: 71–83.
- Hindmarch, I. (2002) Beyond the monoamine hypothesis: mechanisms, molecules and methods. *Eur. Psychiatry*, 17: 294–299.
- Hirst, W.D., Stean, T.O., Rogers, D.C., Sunter, D., Pugh, P., Moss, S.F., Bromidge, S.M., Riley, G., Smith, D.R., Bartlett, S., Heidbreder, C.A., Atkins, A.R., Lacroix, L.P., Dawson, L.A., Foley, A.G., Regran, C.M. and Upton, N. (2006) SB-399885 is a potent, selective 5-HT₆ receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. *Eur. J. Pharmacol.*, 553: 109–119.
- Hoffman, B.J., Hansson, S.R., Mezet, E. and Palkovits, M. (1998) Localisation and dynamic regulation of biogenic amine transporters in the mammalian nervous system. *Front. Neuroendocrinol.*, 19: 187–231.
- Hoyer, D. (2007) RNA interference for studying the molecular basis of neuropsychiatric disorders. *Curr. Opin. Drug Discov. Dev.*, 10: 122–129.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71: 533–554.
- Huang, Y. and Williams, W.A. (2007) Enhanced selective serotonin re-uptake inhibitors as antidepressants: 2004–2006. *Expert Opin. Ther. Patents*, 17: 889–907.
- Ichikawa, J. and Meltzer, H.Y. (1999) R(+)-8-OH-DPAT, a serotonin(1A) receptor agonist, potentiated S(–)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. *J. Pharmacol. Exp. Ther.*, 291: 1227–1232.
- Iversen, L. (2006) Neurotransmitter transporters and their impact on the development of psychopharmacology. *Br. J. Pharmacol.*, 147: 82–88.
- Jayanthi, L.D. and Ramamoorthy, S. (2005) Regulation of monoamine transporters: influence of psychostimulants and therapeutic antidepressants. *AAPS J.*, 7: 728–738.
- Kalueff, A.V., Ren-Patterson, R.F. and Murphy, D.L. (2007) The developing use of heterozygous mutant mouse models in brain monoamine transporter research. *Trends Pharmacol. Sci.*, 28: 122–127.
- Kapur, S., Langlois, X., Vinken, P., Megens, A.A.H.P., De Coster, R. and Andrews, J.S. (2002) The differential effects of atypical antipsychotics on prolactin elevation are explained by their differential blood–brain barrier disposition: a pharmacological analysis in rats. *J. Pharmacol. Exp. Ther.*, 302: 1129–1134.
- Kapur, S. and Mamo, D. (2003) Half a century of antipsychotics and still a central role for dopamine D₂ receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1081–1090.
- Kapur, S., Zipursky, R.B. and Remington, G. (1999) Clinical and theoretical implications of 5-HT₂ and D₂ receptor occupancy of clozapine, risperidone and olanzapine in schizophrenia. *Am. J. Psychiatry*, 156: 286–293.
- Kehne, J.H. (2007) The CRF1 receptor, a novel target for the treatment of depression, anxiety, and stress-related disorders. *CNS Neurol. Disord. Drug Targets*, 6: 163–182.
- Kerwin, R., Millet, B., Herman, E., Banki, C.M., Lublin, H., Pans, M., Hanssens, L., L'Italien, G., McQuade, R.D. and Beuzen, J.-N. (2007) A multicentre, randomized, naturalistic, open-label study between aripiprazole and standard care in the management of community-treated schizophrenic patients (STAR study). *Eur. Psychiatry*, 22: 433–443.
- Kikuchi, T., Tottori, K., Uwahodo, Y., Hirose, T., Miwa, T., Oshiro, Y. and Mortia, S. (1995) 7-[4-[4-(2,3-dichlorophenyl)-1-piper-azinyl]butyloxy]-3,4-dihydro-2(1H)-quinolone (OPC-14597), a new putative antipsychotic drug with both presynaptic dopamine autoreceptor agonistic activity and postsynaptic D₂ receptor antagonistic activity. *J. Pharmacol. Exp. Ther.*, 274: 329–336.
- Kim, S.F., Huang, A.S., Snowman, A.S., Teuscher, C. and Snyder, S.H. (2007) Antipsychotic drug-induced weight gain mediated by histamine H₁ receptor-linked activation of hypothalamic AMP-kinase. *PNAS*, 104: 3456–3459.
- Kroeze, W.K., Hufeisen, S.J., Popadak, B.A., Renock, S.M., Steinberg, S., Ernsberger, P., Jayathilake, K., Meltzer, H.Y. and Roth, B.L. (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacology*, 28: 519–526.
- Lacroix, L.P., Dawson, L.A., Hagan, J.J. and Heidbreder, C.A. (2004) 5-HT₆ receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. *Synapse*, 51: 158–164.
- Landén, M. and Thase, M.E. (2006) A model to explain the therapeutic effects of serotonin reuptake inhibitors: the role of 5-HT₂ receptors. *Psychopharmacol. Bull.*, 39: 147–166.
- Lapin, I.P. and Oxenkrug, G.F. (1969) Intensification of the central serotonergic processes as a possible determinant of the thymoleptic effect. *Lancet*, I: 132–136.
- Lawler, C.P., Prioleau, C., Lewis, M.M., Mak, C., Jiang, D., Schetz, J.A., Gonzalez, A.M., Sibley, D.R. and Mailman, R.B. (1999) Interaction of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology*, 20: 612–627.
- Lawrence, A.J. (2007) Optimisation of anti-psychotic therapeutics: a balancing act? *Br. J. Pharmacol.*, 151: 161–162.
- Leslie, R.A. (2001) Gepirone. *Organon. Curr. Opin. Investig. Drugs*, 2: 1120–1127.
- Li, Z., Hunag, M., Prus, A.J., Dai, J. and Meltzer, H.Y. (2007) 5-HT₆ receptor antagonist SB-399885 potentiates haloperidol and risperidone-induced dopamine efflux in the medial prefrontal cortex or hippocampus. *Brain Res.*, 1134: 70–78.
- Lotufo-Neto, F., Trivedi, M. and Thase, M.E. (1999) Meta-analysis of the reversible inhibitors of monoamine oxidase

- type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacology*, 20: 226–247.
- Lundberg, J., Christophersen, J.S., Petersen, K.B., Loft, H., Halldin, C. and Farde, L. (2007) PET measurement of serotonin transporter occupancy: a comparison of escitalopram and citalopram. *Int. J. Neuropsychopharmacol.*, 10: 777–785.
- Luttgen, M., Elvander, E., Madjidd, N. and Ogren, S.O. (2005) Analysis of the role of 5-HT_{1A} receptors in spatial and aversive learning in the rat. *Neuropharmacology*, 48: 830–852.
- Mace, S. and Taylor, D. (2000) Selective serotonin reuptake inhibitors: a review of efficacy and tolerability in depression. *Exp. Opin. Pharmacother.*, 1: 917–933.
- Maes, M. and Meltzer, H.Y. (2000) The serotonin hypothesis of major depression. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 933–944. <http://www.acnp.org/g4/GN401000094/CH092.html>
- Mailman, R.B. (2007) GPCR functional selectivity has therapeutic impact. *Trends Pharmacol. Sci.*, 28: 390–396.
- Mamo, D., Graff, A., Mizrahi, R., Shammi, C.M., Romeyer, F. and Kapur, S. (2007) Differential effects of aripiprazole on D₂, 5-HT₂, and 5-HT_{1A} receptor occupancy in patients with schizophrenia: a triple tracer PET study. *Am. J. Psychiatry*, 164: 1411–1417.
- Marek, G.J., Martin-Ruiz, R., Abo, A. and Artigas, F. (2005) The selective 5-HT_{2A} receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology*, 30: 2205–2215.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta[b][1,4]diazepino[6,7,1h]indole]: a novel 5-hydroxytryptamine 2C receptor-selective agonist with pre-clinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320: 486–496.
- McEvoy, J.P., Hsiao, J.K. and Lieberman, J.P. for CATIE investigators. (2006) Effects of antipsychotic medications on psychosocial functioning in patients with chronic schizophrenia: findings from the NIMH CATIE study. *Am. J. Psychiatry*, 164: 428–436.
- McIntyre, R.S., Soczynska, J.K., Woldeyohannes, H.O., Alsuwaidan, M. and Konarski, J.Z. (2007) A preclinical and clinical rationale for quetiapine in mood syndromes. *Expert Opin. Pharmacother.*, 8: 1211–1219.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21(2S): 106S–115S.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27: 1159–1172.
- Meyer, J.H., Houle, S., Sagrati, S., Carella, A., Hussey, D.F., Ginovart, N., Goulding, V., Kennedy, J. and Wilson, A.A. (2004) Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Archives of General Psychiatry*, 61(12): 1271–1279.
- Milan, M.J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J. Pharmacol. Exp. Ther.*, 295: 853–861.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. and Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, 78: 189–225.
- Monsma, F.J., Jr., Shen, Y., Ward, R.P., Hamblin, M.W. and Sibley, D.R. (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.*, 43: 320–327.
- Moreau, J.-L., Bos, M., Jenck, F., Martin, J.R., Mortas, P. and Wichman, J. (1996) 5HT_{2C} receptor agonists exhibit antidepressant-like properties in the anhedonia model of depression in rats. *Eur. Neuropsychopharmacol.*, 6: 169–175.
- Muller, M.J. (2007) Gender-specific associations of depression with positive and negative symptoms in acute schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 31: 1095–1100.
- Murphy, B.P., Chung, Y.C., Park, T.W. and McGorry, P.D. (2006) Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. *Schizophr. Res.*, 88: 5–25.
- Natesan, S., Reckless, G.E., Nobrega, J.N., Fletcher, P.J. and Kapur, S. (2006) Dissociation between in vivo occupancy and functional antagonism of dopamine D₂ receptors: comparing aripiprazole to other antipsychotics in animal models. *Neuropsychopharmacology*, 31: 1854–1863.
- Nestler, E.J. and Carlezon, W.A., Jr. (2006) The mesolimbic dopamine reward circuit in depression. *Biol. Psychiatry*, 59: 1151–1159.
- Newman-Tancredi, A., Assie, M.-B., Martel, J.-C., Cosi, C., Bruins Slot, L., Palmier, C., Rauly-Lestienne, I., Colpaert, F., Vacher, B. and Cussac, D. (2007) F15063, a potential antipsychotic with D₂/D₃ antagonist, 5-HT_{1A} agonist and D₄ partial agonist properties: (1) in vitro receptor affinity and efficacy profile. *Br. J. Pharmacol.*, 151: 237–252.
- Nierenberg, A.A. and DeCecco, L.M. (2001) Definitions of antidepressant treatment response, remission, nonresponse, partial response, and other relevant outcomes: a focus on treatment-resistant depression. *J. Clin. Psychiatry*, 62: 5–9.
- Nihalani, N.D. and Schwartz, T.L. (2007) Mifepristone, a glucocorticoid antagonist for the potential treatment of psychotic major depression. *Curr. Opin. Investig. Drugs*, 8: 563–569.
- Owens, M.J., Morgan, W.N., Plott, S.J. and Nemeroff, C.B. (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J. Pharmacol. Exp. Ther.*, 283: 1305–1322.
- Papakostas, G.I., Thase, M.E., Fava, M., Craig Nelson, J. and Shelton, R.C. (2007a) Are antidepressant drugs that combine serotonergic and noradrenergic mechanisms of action more effective than the selective serotonin reuptake inhibitors in treating major depressive disorder? A meta-analysis of studies of newer agents. *Biol. Psychiatry*, 62: 1217–1227.

- Papakostas, G.I., Shelton, R.C., Smith, J. and Fava, M. (2007b) Augmentation of antidepressants with atypical antipsychotic medications for treatment-resistant major depressive disorder: a meta-analysis. *J. Clin. Psychiatry*, 68: 826–831.
- Patkar, A.A., Pae, C.U. and Zarzar, M. (2007) Transdermal selegiline. *Drugs Today (Barc.)*, 43: 361–377.
- Pierre Oli , J. and Kasper, S. (2007) Efficacy of agomelatine, a MT1/MT2 receptor agonist with 5-HT2C antagonistic properties, in major depressive disorder. *Int. J. Neuropsychopharmacol.*, 10: 661–673.
- Rasmussen, K. (2006) Creating more effective antidepressants: clues from the clinic. *Drug Discov. Today*, 11: 623–631.
- Renoldi, G. and Invernizzi, R.W. (2006) Blockade of tachykinin NK1 receptors attenuates stress-induced rise of extracellular noradrenaline and dopamine in the rat and gerbil medial prefrontal cortex. *J. Neurosci. Res.*, 84: 961–968.
- Roberts, J.C., Reavill, C., East, S.Z., Harrison, P.J., Patel, S., Routledge, C. and Leslie, R.A. (2002) The distribution of 5-HT₆ receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT₆ receptor antagonist [¹²⁵I]SB-258585. *Brain Res.*, 934: 49–57.
- Rogers, D. and Hagan, J.J. (2001) 5-HT₆ receptor antagonists enhance retention of a water maze task in the rat. *Psychopharmacology*, 158: 114–119.
- Rosenzweig-Lipson, S., Beyer, C.E., Hughes, Z.A., Khawaja, X., Rajarao, S.J., Malberg, J.E., Rahman, Z., Ring, R.H. and Schechter, L.E. (2007) Differentiating antidepressants of the future: efficacy and safety. *Pharmacol. Ther.*, 113: 134–153.
- Roth, B.L., Sheffler, D.J. and Kroeze, W.K. (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.*, 3: 353–359.
- Rudnick, G. and Clark, J. (1993) From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim. Biophys. Acta*, 1144: 249–263.
- Rush, A.J., Trivedi, M.H., Wisniewski, S.R., Nierenberg, A.A., Stewart, J.W., Warden, D., Niederehe, G., Thase, M.E., Lavori, P.W., Lebowitz, B.D., McGrath, P.J., Rosenbaum, J.F., Sackeim, H.A., Kupfer, D.J., Luther, J. and Fava, M. (2006) Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am. J. Psychiatry*, 163: 1905–1917.
- Sanchez, C. (2006) The pharmacology of citalopram enantiomers: the antagonism by R-citalopram on the effect of S-citalopram. *Basic Clin. Pharmacol. Toxicol.*, 99: 91–95.
- Sanchez, C. and Hyttel, J. (1999) Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol. Neurobiol.*, 19: 467–489.
- Schatzberg, A.F. and Schildkraut, J.J. (2000) Recent studies on norepinephrine systems in mood disorders. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, <http://www.acnp.org/g4/GN401000092/CH090.html>
- Schechter, L.E., McGonigle, P. and Barrett, J.E. (1999) Serotonergic antidepressants: current and future perspectives. *Curr. Opin. CPNS Invest. Drugs*, 1: 432–447.
- Schildkraut, J.J. (1965) The catecholamine hypothesis of affective disorders. *Am. J. Psychiatry*, 122: 509–522.
- Schmidt, C.J., Sorensen, S.M., Kehne, J.H., Carr, A.A. and Palfreyman, M.G. (1995) The role of 5-HT_{2A} receptors in antipsychotic activity. *Life Sciences*, 56(25): 2209–2222.
- Seeman, P. (2006) Targeting the dopamine D₂ receptor in schizophrenia. *Expert. Opin. Ther. Targets*, 10: 515–531.
- Serrats, J., Mengod, G. and Cortes, R. (2005) Expression of serotonin 5-HT_{2C} receptors in GABAergic cells of the anterior raphe nuclei. *J. Chem. Neuroanat.*, 29: 83–91.
- Shaw, A.M., Boules, M., Zhang, Y., Williams, K., Robinson, J., Carlier, P.R. and Richelson, E. (2007) Antidepressant-like effects of novel triple reuptake inhibitors, PRC025 and PRC050. *Eur. J. Pharmacol.*, 555: 30–36.
- Shelton, R.C. (2007) Augmentation strategies to increase antidepressant efficacy. *J. Clin. Psychiatry*, 68: 18–22.
- Skapinakis, P., Papatheodorou, T. and Mavreas, V. (2007) Antipsychotic augmentation of serotonergic antidepressants in treatment-resistant obsessive-compulsive disorder: a meta-analysis of the randomized controlled trials. *Eur. Neuropsychopharmacol.*, 17: 79–93.
- Slatery, D.A., Hudson, A.L. and Nutt, D.J. (2004) Invited review: the evolution of antidepressant mechanisms. *Fundam. Clin. Pharmacol.*, 18: 1–21.
- Sprague, J.E., Everman, S.L. and Nichols, D.E. (1998) An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. *Neurotoxicology*, 19: 427–441.
- Sumiyoshi, T., Matsui, M., Nohara, S., Yamashita, I., Kurachi, M., Sumiyoshi, C., Jayatilake, K. and Meltzer, H.Y. (2001) Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *Am. J. Psychiatry*, 158: 1722–1725.
- Surratt, C.K., Ukaire, O.T. and Ramanujapuram, S. (2005) Recognition of psychostimulants, antidepressants, and other inhibitors of synaptic neurotransmitter uptake by the plasma membrane monoamine transporters. *AAPS J.*, 7: 739–751.
- Tamminga, C.A. (2002) Partial dopamine agonists in the treatment of psychosis. *J. Neural. Transm.*, 109: 411–420.
- Tarsy, D., Baldessarini, R.J. and Tarazi, F.I. (2002) Effects of newer antipsychotics on extrapyramidal function. *CNS Drugs*, 16: 23–45.
- Torres, G.E. and Amara, S.G. (2007) Glutamate and monoamine transporters: new visions of form and function. *Curr. Opin. Neurobiol.*, 17: 304–312.
- Trichard, C., Palliere-Martinot, M.L., Attar-Lvey, D., Recassens, C., Monnet, F. and Martinot, J.L. (1998) Binding of antipsychotic drugs to cortical 5-HT_{2A} receptors: a PET study of chlorpromazine, clozapine and amisulpride in schizophrenic patients. *Am. J. Psychiatry*, 155: 505–508.
- Trivedi, M.H., Fava, M., Wisniewski, S.R., Thase, M.E., Quitkin, F., Warden, D., Ritz, L., Nierenberg, A.A., Lebowitz, B.D., Biggs, M.M., Luther, J.F., Shores-Wilson, K., Rush, A.J. and STAR*D Study Team. (2006) Medication augmentation after the failure of SSRIs for depression. *N. Engl. J. Med.*, 354: 1243–1252.

- Volkow, N.D., Wang, G.J., Fowler, J.S., Learned-Coughlin, S., Yang, J., Logan, J., Schlyer, D., Gatley, J.S., Wong, C., Zhu, W., Pappas, N., Schueller, M., Jayne, M., Carter, P., Warner, D., Ding, Y.S., Shea, C. and Xu, Y. (2005) The slow and long-lasting blockade of dopamine transporters in human brain induced by the new antidepressant drug radafaxine predict poor reinforcing effects. *Biol. Psychiatry*, 57: 640–646.
- Vollenweider, X.F., Vollenweider-Scherpenhuyzen, M.F., Babler, A., Vogel, H. and Hell, D. (1998) Psilocybin induces schizophrenia-like psychosis in humans via serotonin-2 agonist action. *Neuroreport*, 9: 3897–3902.
- White, K.J., Walline, C.C. and Barker, E.L. (2005) Serotonin transporters: implications for antidepressant drug development. *AAPS J.*, 7: 421–433.
- Willner, P. (2000) Dopaminergic mechanisms in depression and mania. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, <http://www.acnp.org/g4/GN401000093/CH.html>
- Wolf, M.E., Deutch, A.Y. and Roth, R.H. (1987) Pharmacology of central dopamine neurons. In: Henn F.A. and DeLisi L.E. (Eds.), *Handbook of Schizophrenia: Neurochemistry and Neuropharmacology of Schizophrenia*, Vol. 2. Elsevier Science B.V., New York, pp. 101–147.
- Wood, M. (2005) Role of the 5-HT_{2C} receptor in atypical antipsychotics: hero or villain? *Curr. Med. Chem. CNS Agents*, 5: 63–66.
- Wood, M.D. (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.*, 2: 383–387.
- Wood, M.D., Heidbreder, C., Reavill, C., Ashby, C.R., Jr. and Middlemiss, D.N. (2001) 5-HT_{2C} receptor antagonist: potential in schizophrenia. *Drug Dev. Res.*, 54: 88–94.
- Wood, M.D. and Reavill, C. (2007) Aripiprazole acts as a selective dopamine D₂ receptor partial agonist. *Expert Opin. Investig. Drugs*, 16: 771–775.
- Wood, M.D., Scott, C., Clarke, K., Cato, K.J., Patel, N., Heath, J., Worby, A., Gordon, L., Campbell, L., Riley, G., Davis, D.N.C., Gribble, A. and Jones, D.N.C. (2006) Pharmacological profile of antipsychotics at monoamine receptors: atypicality beyond 5-HT_{2A} receptor blockade. *CNS Neurol. Disord. Drug Targets*, 5: 445–452.
- Woolley, M.L., Marsden, C.A. and Fone, K.C.F. (2004) 5-HT₆ receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3: 59–79.
- Yamada, M. and Yasuhara, H. (2004) Clinical pharmacology of MAO inhibitors: safety and future. *Neurotoxicology*, 25: 215–221.
- Yokoi, F., Grunder, G., Biziere, K., Stephane, M., Dogan, A.S., Dannals, R.F., Ravert, H., Suri, A., Bramer, S. and Wong, D.F. (2002) Dopamine D₂ and D₃ receptor occupancy in normal humans treated with the antipsychotic drug aripiprazole (OPC 14597): a study using positron emission tomography and [¹¹C]raclopride. *Neuropsychopharmacology*, 27: 248–259.
- Youdim, M.B. and Bakhle, Y.S. (2006) Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. *Br. J. Pharmacol.*, 147: 287–296.
- Zangen, A., Nakash, R., Overstreet, D.H. and Yadid, G. (2001) Association between depressive behavior and absence of serotonin–dopamine interaction in the nucleus accumbens. *Psychopharmacology*, 155: 434–439.
- Zarate, C.A., Jr., Singh, J.B., Carlsson, P.J., Quiroz, J., Jolkovsky, L., Luckenbaugh, D.A. and Manji, H.K. (2007) Efficacy of a protein kinase C inhibitor (tamoxifen) in the treatment of acute mania: a pilot study. *Bipolar Disord.*, 9: 561–570.
- Zhou, Z., Zhen, J., Karpowich, N.K., Goetz, R.M., Law, C.J., Reith, M.E. and Wang, D.N. (2007) LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. *Science*, 317: 1390–1393.
- Zhu, C.B., Carneiro, A.M., Dostmann, W.R., Hewlett, W.A. and Blakely, R.D. (2005) p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J. Biol. Chem.*, 280: 15649–15658.

CHAPTER 12

The dorsal raphe nucleus and serotonin: implications for neuroplasticity linked to major depression and Alzheimer's disease

Kimmo A. Michelsen, Jos Prickaerts and Harry W.M. Steinbusch*

*Department of Neuroscience, Faculty of Health, Medicine and Life Sciences, Maastricht University;
European Graduate School of Neuroscience (EURON), PO Box 616, 6200 MD Maastricht, The Netherlands*

Abstract: The dorsal raphe nucleus (DRN) is a heterogeneous brainstem nucleus located in the midbrain and pons. Via widespread projections, which target a multitude of brain areas, its neurons utilize many transmitters to control various physiological functions, including learning, memory and affect. Accordingly, the DRN has been strongly associated with brain dysfunction, especially mood disorders such as depression, but also Alzheimer's disease. The DRN's most abundant transmitter, serotonin, has received the most attention in studies on both normal brain function and disease, and lately its involvement in the regulation of neuroplasticity has been under particular scrutiny. This chapter begins with a systematic overview of what we currently know about the anatomy of the DRN and its neurons, including their ascending projections. It continues with a review of the transmitters of the DRN, followed by a discussion on the connection between the DRN and neuroplasticity. Special emphasis is put on serotonin and its central role in neuroplasticity, which is proving to be of high priority in unraveling the full picture of the cellular mechanisms and their interconnections in the etiology of major depression and Alzheimer's disease.

Keywords: dorsal raphe nucleus; serotonin; neuroplasticity; major depression; Alzheimer's disease

Introduction

The dorsal raphe nucleus (DRN) is a bilateral, heterogeneous brainstem nucleus. It is located in the ventral periaqueductal grey matter of the mesencephalon, with a caudal tip reaching into the pons. The neurons of the DRN innervate a multitude of targets throughout the brain and utilize many transmitters, of which serotonin is the most abundant and important one. The DRN is involved in the control

of various physiological functions and has been implicated in brain dysfunction, especially mood disorders such as depression. This chapter gives an overview of the current knowledge about the DRN with emphasis on its neurons, transmitters and ascending projections and on its role in depression and Alzheimer's disease (AD).

DRN morphology

The DRN is a bilateral, heterogeneous brainstem nucleus situated in the midbrain and the pons. Most of its cells are located in the ventral part of

*Corresponding author. Tel.: +31-43-3881021;
Fax: +31-43-3671096; E-mail: h.steinbusch@np.unimaas.nl

the periaqueductal grey matter of the midbrain. The nucleus' most rostral part is at the level of the oculomotor nucleus, whereas its caudal tip lies in the periventricular grey matter of the rostral pons. From the 1960s to the early 1980s, the morphology of the DRN was described in the cat (Taber et al., 1960), man (Braak, 1970), the rabbit (Felten and Cummings, 1979) and the rat (Steinbusch, 1981). Together with the caudal linear and median raphe nucleus, the DRN forms the rostral or superior division of the raphe complex. The caudal or inferior division encompasses the raphe obscurus, raphe pallidus and raphe magnus nuclei and parts of the lateral reticular formation, located in the medulla and caudal pons (Steinbusch, 1981; Jacobs and Azmitia, 1992).

Serotonin is the major neurotransmitter of the DRN. The morphology of the serotonergic system in the DRN was first described in the rat by Dahlström and Fuxe (1964), using formaldehyde-induced fluorescence (FIF) which had been developed by Falck et al. (1962) for visualization of monoamines. The human DRN has been estimated to contain $235,000 \pm 13,000$ neurons (Baker et al., 1990), of which approximately $165,000 \pm 34,000$ (or $70\% \pm 14\%$) neurons contain serotonin (Baker et al., 1991). In the cat, the DRN has been estimated to contain 35,000 neurons, of which up to 70–80% are serotonergic, as demonstrated by the FIF technique (Wiklund et al., 1981; Leger and Wiklund, 1982). The rat DRN has been estimated to contain approximately 35,000 neurons, of which about one-third are serotonergic (Descarries et al., 1982). This inconsistency across cat and rat studies may be due to methodological differences, since the rat results were based on the measured uptake of tritiated serotonin, and only medium-sized non-indolaminergic neurons were counted in the cat studies.

According to the original nomenclature by Dahlström and Fuxe, the raphe nuclei (including the brainstem reticular formation) are divided into nine subdivisions, B1–B9. The subdivisions were later renamed and slightly redefined when the nuclei were re-examined using an antibody against serotonin, and what is now considered as the DRN corresponds to the original subdivisions B6–B7, B6 being the caudal extension. In most

species, the DRN can be divided into five subregions, namely the interfascicular, ventral (or ventromedial), ventrolateral (or lateral), dorsal and caudal subregions (Baker et al., 1990). The DRN is also often divided along the rostrocaudal axis into a rostral, middle and caudal portion. All five subregions extend from the rostral to the middle part of the nucleus, except for the caudal subregion, which is located in the caudal portion of the DRN. Abrams et al. (2004) have proposed detailed stereotaxic coordinates for the boundaries of the rostral, middle and caudal portion. Accordingly, the rat and mouse DRN were divided in three equally long parts along the rostrocaudal axis. The rostral part of the rat DRN comprises levels from -6.92 to -7.64 , the middle portion levels from -7.73 to -8.45 and the caudal portion levels from -8.54 to -9.26 mm bregma. In mouse, the proposed corresponding values are -4.12 to -4.48 , -4.54 to 4.90 and -4.96 to -5.32 mm bregma. For both species, values are based on stereotaxic atlases (Paxinos and Watson, 1997; Paxinos and Franklin, 2001) and the authors' own immunostainings with tryptophan hydroxylase (TPH) (Abrams et al., 2004). These coordinates deal with the rostrocaudal axis only, but division into the five subregions is fairly easy to make on a morphological basis.

Neuron types

In human, the DRN contains four main morphological neuronal types (\emptyset = average mean diameter) round (\emptyset , $27 \pm 4 \mu\text{m}$), ovoid (\emptyset , $27 \pm 4 \mu\text{m}$; variance $20\text{--}39 \mu\text{m}$), fusiform (\emptyset , $18 \pm 6 \mu\text{m}$; variance $9\text{--}37 \mu\text{m}$) or triangular ($13 \pm 2 \mu\text{m}$) cells (Baker et al., 1990). In rat, they appear similar and have been described as either small round (\emptyset , $10 \pm 3 \mu\text{m}$), medium-sized fusiform and bipolar (\emptyset , $24 \pm 4 \mu\text{m}$ and 8 ± 4 , respectively), large fusiform (\emptyset , 18 ± 2 and length $31 \pm 2 \mu\text{m}$) and very large multipolar (\emptyset , $39 \pm 5 \mu\text{m}$) (Steinbusch et al., 1981; Steinbusch, 1984).

The four main morphologically different types of DRN neurons are differentially distributed within the DRN, which seems to reflect neurochemical and

functional specialization. Indeed, an increasing number of studies have supported this notion. Electrophysiological studies in the 1980s led to a division of rat serotonergic DRN neurons into two types, which were named Type I and Type II (or typical and atypical serotonergic neurons, respectively). Type I neurons exhibited a rhythmic firing pattern and were called clock-like neurons, whereas Type II neurons fired irregularly and were called non-clock-like (Nakahama et al., 1981). More recently, each type was divided into three distinct classes based on firing patterns during the sleep–wake cycle as measured by single-unit recordings in cats. In addition, non-serotonergic DRN neurons were divided into three groups as well (Sakai and Crochet, 2001).

Properties

Classes I-A and *I-B* displayed a regular firing pattern during waking. During waking, discharge rates were higher than during slow-wave-sleep (SWS), whereas almost no firing occurred during paradoxical sleep (PS, also known as rapid eye movement (REM) sleep). *I-C* neurons differed from *I-A* and *-B* by maintaining a fairly high level of tonic, rhythmic activity during SWS and PS. *Class II-A* neurons' irregular and high discharge rates correlated with motor activity and was high during active wakefulness (AW; presence of gross body movements), feeding and grooming, whereas rates were significantly lower or absent during quiet wakefulness (QW; absence of gross body movements), SWS and PS. *Class II-B* neurons displayed their highest rate of tonic activity during deep SWS and very low rates during PS, as well as AW and QW, but were strongly activated during feeding and grooming. *II-C* fired irregularly and slowly during waking periods of no physical activity and was reduced during PS (Sakai and Crochet, 2001).

In addition, non-serotonergic neurons were identified, and divided into three groups: Type I-S, Type I-R and phasic neurons. I-R and phasic neurons have a brief action potential and fast firing rate, whereas I-S neurons have a similar discharge activity as serotonergic neurons except

that their tonic increase during PS compared to SWS (Sakai and Crochet, 2001).

Distribution

About two-thirds of presumed serotonergic neurons were confined to classes *I-A* and *I-B*, which were evenly distributed throughout the DRN. They seemed to be identical to previously identified serotonergic neurons in cat. Only 6% of serotonergic neurons were confined to Type *I-C*, and were mainly located in the ventral region of the DRN. Type *II-A* neurons were preferentially located in the middle parts and Type *II-B* neurons preferentially in the most rostral and dorsal parts of the DRN. Type *II-C* neurons were located in the ventral portion of the DRN, close to the medial longitudinal bundle and the nucleus annularis. Each class of Type II neurons constituted 8–12% of all serotonergic neurons. To summarize, the clock-like Class I neurons, which are generally waking-dependent, comprise almost three-fourths of serotonergic DRN neurons, whereas the non-clock-like Class II neurons, which are generally motor-dependent, make up less than one-fourth (Sakai and Crochet, 2001).

Projections

Efferent projections of the DRN

Serotonergic neurons of the DRN display a topographic organization along the rostrocaudal axis, with respect to efferent projections (Abrams et al., 2004). Thus, neurons located more rostrally project to more rostral areas of the brain than neurons located more caudally in the DRN.

Yet, individual neurons seem to project to several distinct but functionally related targets through branched fibres (Lowry, 2002). The first branched projections to be discovered run from the dorsal DRN along the dorsal raphe cortical tract to the substantia nigra (SN) and caudate-putamen (CP) (van der Kooy and Hattori, 1980a; Imai et al., 1986). Also, single neurons have been observed to target hippocampus and entorhinal cortex (Kohler and Steinbusch, 1982), prefrontal

cortex and nucleus accumbens (NA) (Van Bockstaele et al., 1993), the paraventricular nucleus (PVN) of the thalamus and the lateral parabrachial nucleus (PBN) (Petrov et al., 1992), the central nucleus of the amygdala (CeA) and the PVN (Petrov et al., 1994), distinct sites in the trigeminal somatosensory pathway (Kirifides et al., 2001) and the vestibular nuclei and CeA (Halberstadt and Balaban, 2006).

This could be a key to understanding the role of the DRN as a modulator of complex autonomic functions with anatomical correlates in several parts of the brain. For instance, both the CeA and the PVN, which are targeted by the same branched fibres, are involved in anxiety and conditioned fear (Petrov et al., 1992, 1994). These fibres emerge from well-defined subpopulations of neurons in the medial part of the middle DRN as well as more caudal clusters.

However, only a part of the neurons with branched axons contain serotonin, the reported range being between 8% (Petrov et al., 1992) and 64% (Halberstadt and Balaban, 2006) depending on the targets. This serves as a reminder that serotonin is not the only transmitter utilized by the DRN. For instance, the CeA-PVN projecting subpopulations mentioned above (where about half the neurons are serotonergic) also contain corticotropin-releasing factor (CRF), which has been associated with anxiety and other mood disorders. Anxiety-related behavioural changes induced by serotonergic activity, such as development of learned helplessness, seem to be CRF-dependent (Maier and Watkins, 2005). However, it has not been shown, whether the CRF-containing neurons themselves, or the serotonergic DRN neurons they target, send collaterals to CeA and PVN.

Early studies showed that most DRN neurons project ipsilaterally and few contralaterally (Miller et al., 1975). Retrograde labelling studies of DRN efferents to the entorhinal cortex indicated that, when present, contralateral terminals are preferentially located close to the midline (Kohler and Steinbusch, 1982). Similar results were obtained recently in a study by Waselus and co-workers, in which all DRN neurons, which sent collaterals to lateral septum and striatum, were located

ventromedially near the midline or slightly lateral to it. Notably, all such collateral neurons were serotonergic (Waselus et al., 2006). However, single neurons do not seem to project collaterally to both hemispheres (van der Kooy and Hattori, 1980b; Kohler and Steinbusch, 1982). Besides their topographic organization, different cell types also seem to display different projections. This has, however, not been extensively studied but is reflected in the distribution patterns of different cell types versus the projections emerging from different areas.

Fibre morphology

Fibres arising from the DRN are characteristically very fine and have small varicosities, which are granular or fusiform in shape (so-called type D axons). This is in contrast to fibres arising from the medial raphe nuclei (MRN), which display large, spherical varicosities (so-called type M axons) and variations in fibre thickness (Kosofsky and Molliver, 1987). Serotonin-immunoreactive fibres display similar variation, which may help to give an indication of the origin of serotonergic fibres in purely immunohistochemical preparations (Kosofsky and Molliver, 1987; Mulligan and Tork, 1988). At the light microscopic level, three types of serotonergic axons can be distinguished. Of these, one arises from the DRN while two are continuous with each other and arise from the MRN: (1) fine (less than 0.5 μm) DRN fibres with small fusiform varicosities (generally less than 1 μm in diameter); (2) thick (about 1 μm) and smooth non-varicose MRN fibres, which travel long distances in straight trajectories; (3) shorter and thinner varicose fibres (varicosity size 1 μm or more in diameter) arise from the thick MRN fibres. The thinner DRN fibres branch frequently and target large, often diffuse areas, whereas the thicker fibres branch infrequently, and are often seen to surround the somata of single neurons (Mulligan and Tork, 1988). On an electron microscopic level, the DRN fibres display small, fusiform boutons and are believed to signal predominantly via volume transmission, whereas the MRN fibres contact their target via large round boutons, often in large numbers (Tork, 1990). The morphology and origin

of the fibres has also been linked to differential drug-sensitivity, first demonstrated in the fore-brain, where the neurotoxic amphetamine derivatives methylenedioxymphetamine (MDA) and *p*-chloroamphetamine (PCA) induce denervation of the fine axons, whereas the thick ones are unaffected by the drugs (Mamounas and Molliver, 1988; O'Hearn et al., 1988; Mamounas et al., 1991). A suggested reason for this difference is serotonin transporter (SERT) expression, which, in amygdala, is present in the thick MR drug-insensitive fibres but lacking from the thin DRN drug-sensitive ones (Brown and Molliver, 2000).

Thus, functionally the serotonergic fibres seem to be organized into two main subsystems, of which the DRN system has a more widespread influence via its highly divergent branches and volume transmission, while the MRN system has extensive and direct synaptic contacts with neuronal somata.

Pathway overview

The DRN projects along several ascending and descending pathways, most of which it shares with one or more of the other raphe nuclei. The

pathway nomenclature differs slightly between authors and the division is not completely consistent, with some overlaps and contradictions, especially in the older literature. In this review, we have labelled the pathways according to Steinbusch et al.

Ascending pathways

There are three ascending pathways: the dorsal, medial and ventral ascending pathways (Fig. 1). Of these, the dorsal and ventral ascending pathways are the two most important efferent projections of the DRN. They reach a multitude of targets throughout the forebrain, the most important one being the CP.

Descending pathways

In addition, four descending projections leave the DRN: the bulbospinal pathway, cerebellar pathway, propriobulbar pathway and one that innervates the locus coeruleus, dorsal tegmental nucleus and pontine raphe nucleus. The main targets of the descending pathways are cerebellum, the lower

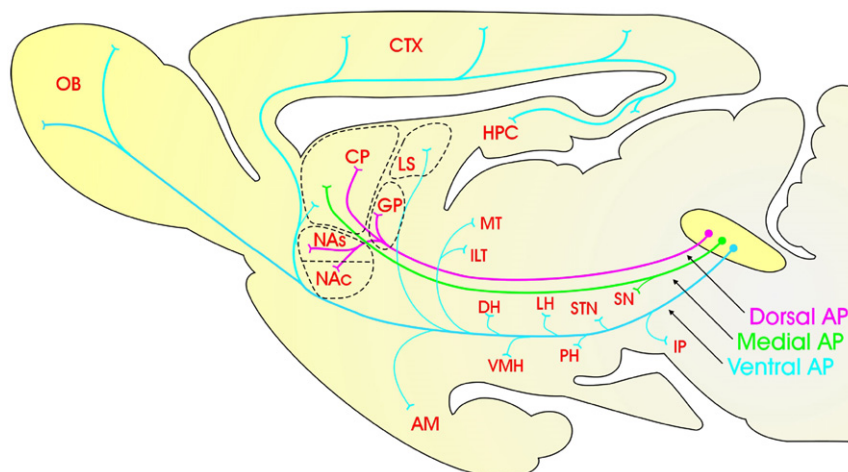


Fig. 1. The three ascending pathways (APs) of the rat DRN and their main targets. AM, amygdala; CP, caudate-putamen; CTX, cortex; DH, dorsal hypothalamus; GP, globus pallidus; HPC, hippocampus; ILT, intralaminar thalamic nuclei; IP, interpeduncular nucleus; LH, lateral hypothalamus; LS, lateral septum; MT, midline thalamic nuclei; NAs, shell of nucleus accumbens; NAc, core of nucleus accumbens; SN, substantia nigra; STN, subthalamic nucleus; PH, posterior hypothalamus; VMH, ventromedial hypothalamus. (Reprinted from Michelsen et al., 2007, with permission from Elsevier) (See Color Plate 12.1 in color plate section.)

brainstem and the spinal chord. These pathways will not be further dealt with in this chapter.

The dorsal ascending pathway

The dorsal ascending pathway rises from medial and rostral DRN. Eighty per cent of its fibres are serotonergic. It innervates the striatum and globus pallidus (GP).

Striatum

Anterograde labelling has shown that DRN efferents target the ventromedial striatum at caudal to midlevel (Vertes, 1991). Most striatum-projecting neurons are located in rostral DRN (Steinbusch et al., 1981). Recent retrograde labelling studies confirmed a gradient of striatum-projecting neurons in the DRN. Approximately half of the neurons were located in the rostral third of the DRN. Three out of eight neurons were seen in middle DRN, parallel to the midline, with highest concentrations dorsomedially and ventrally. Only one out of eight neurons were located in caudal DRN, in its dorsomedial part (Waselus et al., 2006).

The CP is extensively innervated by neurons of the DRN. It is the single most important of targets for DRN innervation and one of the first to be extensively studied. The earliest anatomical indications for DRN projections to the CP (Anden et al., 1965) were subsequently supported by lesion studies, which showed a drop in striatal TPH activity (Geyer et al., 1976) as well as a decrease in [³H]5-HT uptake (Kellar et al., 1977) after DRN lesions, and by in vivo microdialysis, which showed that electrical stimulation of the DRN lead to a rise in serotonin dialysate in the CP (McQuade and Sharp, 1997). Meanwhile, more anatomical data has accumulated. Approximately one-third of all serotonergic DRN neurons project to the CP. This is, however, region-specific: in a cluster in dorsomedial DRN, 80–90% of serotonergic neurons were found to project to the CP (Steinbusch et al., 1981). In addition, 80% of DRN neurons that project to the CP are serotonergic, and they mainly project ipsilaterally. The remaining 20% of non-serotonergic CP-projecting

neurons are mostly found in the caudal parts of ventromedial and dorsomedial DRN (see Fig. 2 for an overview of DRN anatomy).

The innervation of NA is even higher than that of the CP. A majority of the innervation is serotonergic. The shell of the NA is more heavily innervated than the core, especially in more caudal regions where the fibres in the shell are much more abundant than elsewhere in the NA. The core is innervated exclusively by thin (0.3 µm) smooth axons, similar to the rostral shell, which is innervated predominantly by thin axons and, to a lesser extent, by varicose fibres. In contrast, the caudal shell is innervated predominantly by thicker, highly varicose (0.5 µm between varicosities) serotonergic axons (Van Bockstaele and Pickel, 1993; Brown and Molliver, 2000). It has not been determined that all the innervation indeed stems from the DRN. However, studies on projections to cerebral cortex and olfactory bulb have shown that thin drug-sensitive serotonin axons typically arise from the DRN and varicose, drug-resistant axons arise from the MRN (Kosofsky and Molliver, 1987; Mamounas et al., 1991). In NA, the thin fibres of the core are more vulnerable to amphetamine derivatives than the thick fibres of the shell, indicating that the DRN innervates the NA core, as suggested by Brown and Molliver (2000).

Globus pallidus

Pallidal afferents from DRN have been demonstrated by tracing studies. Vertes (1991) used the retrograde tracer PHA-L in rats and DeVito et al. (1980) used the anterograde tracer HRP in macaque monkeys. The innervation of GP is mainly serotonergic, as confirmed by microdialysis studies in the rat, where stimulation of the DRN increased serotonin dialysate in the GP by 75%. In the same study, stimulation of the MRN had little or no effect (McQuade and Sharp, 1997).

Medial ascending pathway

The main target of the medial ascending pathway is SN. To a lesser extent, the pathway also

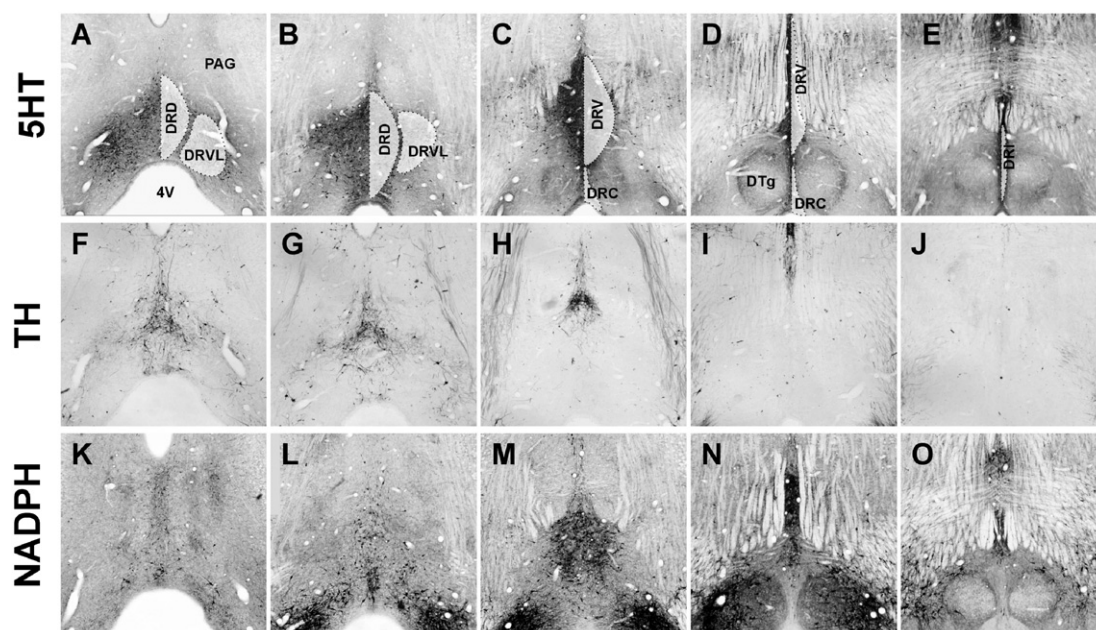


Fig. 2. Three of the transmitters of the DRN, serotonin, dopamine and nitric oxide visualized in horizontal sections of mouse brain with DAB-immunohistochemistry against serotonin (first row), tyrosine hydroxylase (second row) and NADPH diaphorase (third row), respectively. The delineation of the subdivisions is based on the Paxinos mouse brain atlas (Paxinos and Franklin, 2001). The images in each column are from adjacent sections. The left column shows the most dorsal sections and the right column the most ventral ones. DRC, caudal DRN; DRD, dorsal DRN; DRI, infralimbic DRN; DRV, ventral DRN; DRVL, ventrolateral DRN; DTg, dorsal tegmental nucleus; PAG, periaqueductal grey; 4V, fourth ventricle.

innervates the CP. The fibres emerge from rostral parts of the DRN (van der Kooy and Hattori, 1980a; Imai et al., 1986).

Substantia nigra

Studies with the retrograde tracer HRP injected to the SN showed labelled neurons in the DRN but not in the MRN (Bunney and Aghajanian, 1976; Fibiger and Miller, 1977). The projections seem to arise from rostral DRN (Imai et al., 1986) and they target the pars compacta division in particular, whereas the pars reticulata seems to be innervated to a lesser, yet substantial degree, as indicated by studies using [^3H]leucine (Fibiger and Miller, 1977) and [^{14}C]leucine (Bobillier et al., 1976) injections into the DRN. However, a study using the retrograde tracer PHA-L failed to demonstrate DRN innervation of the pars reticulata (Vertes, 1991).

Caudate-putamen

Although the dorsal ascending pathway conveys most of the raphe input to the CP, it also receives innervation via the medial ascending pathway. Some of the fibres branch and target both the SN and CP (van der Kooy and Hattori, 1980a; Imai et al., 1986). Thus, single DRN neurons can exert control over both the SN and the CP.

Ventral ascending pathway

Via the ventral ascending pathway, the DRN innervates many areas. The bilateral pathway ascends ventrolaterally and then turns rostrally to enter the medial forebrain bundle. The pathway also contains fibres from other raphe nuclei, especially the MRN. The main targets are thalamic and hypothalamic nuclei, habenula, septum, amygdala, cortex, the olfactory bulb, hippocampus, interpeduncular nucleus and geniculate body.

Hypothalamus

Several studies have addressed the projections from the raphe nuclei to the hypothalamus. In an early autoradiographic study, in which [^{14}C] tracing was used to map DRN efferents in cat, Bobillier et al. (1976) identified varying degrees of DRN innervation in several hypothalamic nuclei. Most of these results were later confirmed, while some have been contradicted by subsequent experiments. In addition, some studies have not distinguished between the raphe subnuclei. For instance Steinbusch and Nieuwenhuys (1982) demonstrated serotonergic innervation in nearly all parts of the hypothalamus, but they did not attempt to locate the projecting perikarya. Some studies have indicated that the MRN is a greater source of hypothalamic serotonin innervation than the DRN (Geyer et al., 1976; Kellar et al., 1977).

The DRN has been reported to innervate the SCN and preoptic area (Bobillier et al., 1976), but later studies in the rat suggest that DRN does not project to these structures (van de Kar and Lorens, 1979; Meyer-Bernstein and Morin, 1996). The dense serotonergic innervation of the SCN and medial preoptic area (Bobillier et al., 1976), and the light-to-moderate serotonergic innervation of the rest of the anterior hypothalamus, seems to emerge from the MRN instead (van de Kar and Lorens, 1979; Hay-Schmidt et al., 2003).

Tracings studies have identified moderate DRN innervation in posterior hypothalamus (Bobillier et al., 1976) and lesion studies have shown that the arcuate nucleus receives innervation from DRN (van de Kar and Lorens, 1979). Furthermore, the lateral hypothalamus receives high innervation from the DRN. This was shown by early [^{14}C] tracing studies, such as by Bobillier et al. (1976) and later confirmed with PHA-L tracing (Vertes, 1991). A recent anterograde tracing study showed that neurons in the central portion of the rostral DR innervate about 23% of the orexinergic neurons of the lateral hypothalamus, mainly in the lateral parts of the cluster (Yoshida et al., 2006).

Thalamus

Several of the thalamic nuclei receive innervation from the DRN. Studies in cat and rat have reported dense innervation in the midline and intralaminar nuclei of the thalamus (including the posterior paraventricular, the parafascicular, reuniens, rhomboid, intermediodorsal/mediodorsal and central medial thalamic nuclei) and moderate innervation in thalamic paracentral and central lateral intralaminar nuclei (Conrad et al., 1974; Bobillier et al., 1976; Vertes, 1991). In addition, the subparafascicular and prethalamic nuclei (Bobillier et al., 1976) have been reported to receive innervation from the DRN, but confirmation by later studies is lacking.

Habenula

The DRN innervates the lateral habenula to a moderate extent, whereas the medial habenula does not seem to receive any innervation in rat (Sim and Joseph, 1993), cat (Bobillier et al., 1976) and hamster (Morin and Meyer-Bernstein, 1999). One study, however, reported low innervation in the medial habenula of rat (Morin and Meyer-Bernstein, 1999). In the same study the hamster lateral habenula was shown to receive only sparse serotonergic innervation, indicating that the input from DRN is mainly non-serotonergic.

Septum

The DRN sends strong innervation to the lateral septum, 80% of which is serotonergic (Kohler et al., 1982). The innervation predominantly targets the medial portions of the lateral septum (Vertes, 1991). Most of the projecting neurons are located throughout the caudal DRN. In the rest of the DRN, neurons are sparse and located ventromedially. The neuron number decreases towards the mid-DRN and is very low in rostral DRN, while most of the rostral parts contain no septum-projecting neurons at all (Waselus et al., 2006). The medial septum is not generally

considered a target of DRN innervation. Microdialysis studies have, however, shown that stimulation of DRN can increase serotonin dialysate in medial septum by more than 55%. This suggests that the DRN does indeed target the area, but as long as anatomical evidence is lacking it can not be excluded that such measurements actually sample serotonin from the lateral septum (McQuade and Sharp, 1997).

Amygdaloid complex

Studies using neuronal tracers, PHA-L in particular, have demonstrated that the basolateral and lateral amygdaloid nuclei, as well as the extended amygdala (*comprising centromedial amygdala + bed nucleus of stria terminalis and substantia innominata, as defined by Alheid and Heimer, 1988*) receive dense innervation from the DRN (Grove, 1988; Vertes, 1991). Also, immunohistochemical techniques in rat have shown that the basolateral amygdaloid nuclei receive strong serotonergic innervation, especially the rostral and medial parts of the basal nucleus, while the caudal part of the basal nucleus as well as the entire lateral nucleus receive a lower, yet high density of serotonin innervation. In the centromedial nuclei innervation is very low, except for the posterior part of the medial amygdaloid nucleus and the medial and lateral parts of the posterior nucleus (Steinbusch, 1981). The serotonin-immunoreactivity in the amygdaloid has not been directly correlated to DRN efferents. However, in squirrel monkeys, the most abundant serotonergic fibre type is thin, with fusiform or pleiomorphic varicosities, which suggests that serotonergic innervation emerges predominantly from the DRN (Sadikot and Parent, 1990). A more recent immunohistochemical study in macaque monkeys is not consistent with the rat data, with regard to the relative fibre density in amygdaloid subnuclei, probably due to species differences. The highest levels were present in lateral subregions of the central amygdala and dorsolateral bed nucleus of stria terminalis. Levels were high in basal amygdala and moderate in centromedial amygdaloid nuclei (Freedman and Shi, 2001).

Cerebral Cortex

Several studies have dealt with cortical projections of the DRN (Bobillier et al., 1976; O'Hearn and Molliver, 1984; Vertes, 1991). O'Hearn and Molliver demonstrated that the cortical projections of rat DRN emerge predominantly from the ventral subnucleus, in particular from immediately dorsal or medial to the medial longitudinal fasciculi. These areas account for three-fourths of the DRN innervation of the cortex, whereas the dorsal subnucleus contributes one-fourth. Along the rostrocaudal axis, most neurons are located in the middle DRN, and the lateral areas of the DRN do not seem to project to the cerebral cortex at all. More than 80% of the projections are serotonergic (O'Hearn and Molliver, 1984). The ratio of contralateral fibres is 26–35%, and differs between the subnuclei. At least in entorhinal cortex, the contralateral fibres seem to preferentially target medial areas (Kohler and Steinbusch, 1982; O'Hearn and Molliver, 1984).

The frontal cortex receives most of its serotonergic innervation from the DRN (Kosofsky and Molliver, 1987). The density is highest in the dorsal frontal cortex and low in caudal regions, with intermediate densities in areas in between (Steinbusch, 1981). The frontal cortex receives projections from nearly twice as many DRN neurons as either the parietal or occipital cortex (O'Hearn and Molliver, 1984). The entorhinal cortex is targeted by both serotonergic and non-serotonergic projections (Segal, 1977) and (Kohler and Steinbusch, 1982) which for the most part emerge from the DRN (Kohler and Steinbusch, 1982). In addition, anterograde labellings with PHA-L have shown that many cortical regions receive dense (the piriform, insular and frontal cortices) or moderately dense (occipital, entorhinal, perirhinal, frontal orbital, anterior cingulate and infralimbic cortices) projections from the DRN (Vertes, 1991) (Fig. 3).

Hippocampus

DRN projects to the hippocampus (Segal and Landis, 1974; Azmitia and Segal, 1978). DRN efferents to the hippocampus emerge predominantly

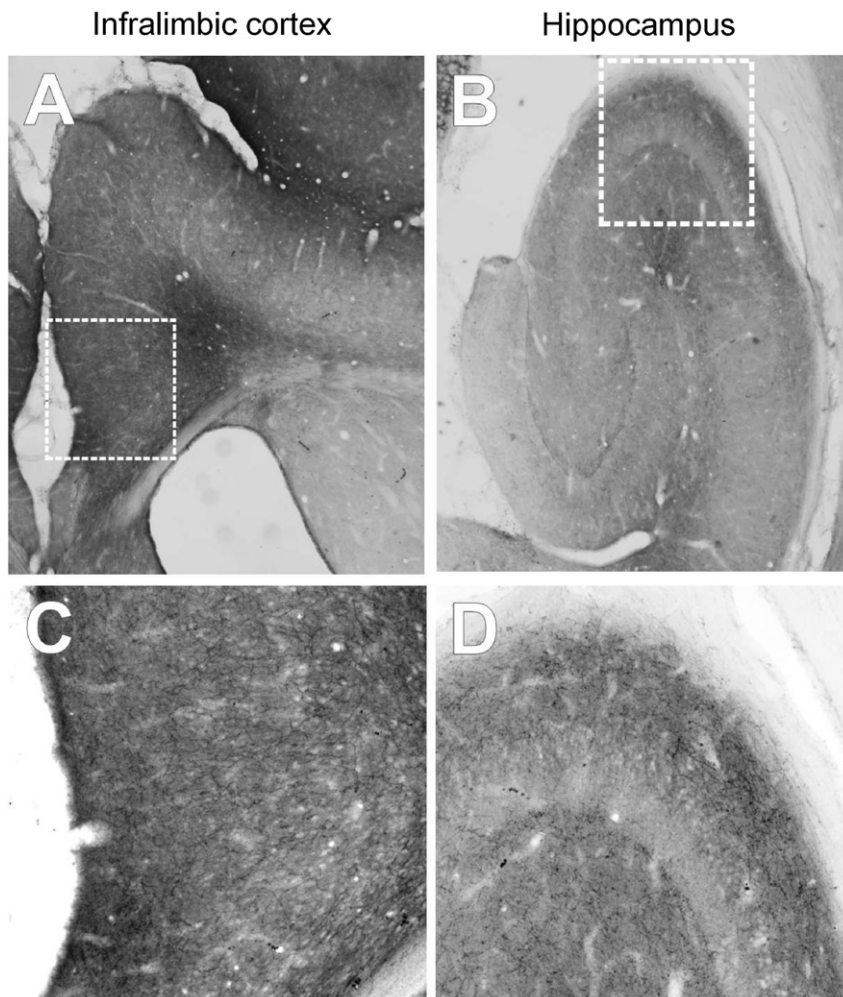


Fig. 3. The serotonergic DRN innervation of medial prefrontal cortex (A) and hippocampus (B) are of special interest with regard to the role of serotonin in depression and AD, respectively. Serotonin was visualized with DAB-immunohistochemistry in coronal sections of mouse brain, (C) and (D) are details of A and B, respectively.

from the most caudal parts of the nucleus, close to the midline, and is both serotonergic and non-serotonergic (Wyss et al., 1979; Kohler and Steinbusch, 1982). Immunohistochemical stainings have demonstrated fine serotonergic axons with small varicosities throughout the hippocampus (Fig. 3). The fibres' morphology (Kosofsky and Molliver, 1987) suggests that they derive from the DRN (Mamounas et al., 1991). However, lesion studies have indicated that the MRN and not the DRN is the major source of hippocampal serotonin innervation (van de Kar and Lorens, 1979).

Olfactory bulb

Tracing studies with radioactively labelled amino acids in rat (Halaris et al., 1976) and cat (Bobillier et al., 1976) have demonstrated DRN projections to the olfactory bulb. The DRN is the primary source of serotonin in the olfactory bulb, as shown by retrograde transport of [3 H]serotonin (Araneda et al., 1980a, b). Immunohistochemical stainings have demonstrated serotonergic innervation of all layers of the olfactory bulb, especially the glomerular lamina (Steinbusch, 1981).

Supraependymal plexus

The supraependymal plexus is a network of serotonergic fibres, which covers nearly all ventricular surfaces with moderate or high density. They are most numerous in the third ventricle and the foramina of Monro, fewer in the lateral ventricles and aqueduct and numerous in the hypothalamic region of the third ventricle. Areas with low density or absence of fibres are the third ventricle floor, the preoptic area, the roof of interventricular foramen, the subfornical organ and the roof of the fourth ventricle (Richards et al., 1973; Chan-Palay, 1976; Lorez and Richards, 1982). The plexus was discovered already in the 1920s (Lorez and Richards, 1982) and later identified as being mainly serotonergic (Richards et al., 1973).

Several studies have indicated that the supraependymal serotonergic fibres ascend from the medial and in particular, dorsal raphe: in rats, supraependymal fibres degenerated after lesions of the dorsal and medial raphe (Aghajanian and Gallager, 1975), and electrical stimulation of the medial and pontine raphe led to an increase in [³H]5-HT uptake from the intracerebroventricular space (Chan-Palay, 1976). Also [¹²⁵I] tetanus toxin was injected into the lateral ventricles labelled neurons of the medial and dorsal raphe by means of retrograde transport along the fibres (Richards, 1978). Further, there is a direct fibre pathway between the DRN and the aqueduct surface in rats (Steinbusch et al., 1981), and in mice, an exit zone for fibres to the fourth ventricle has been reported immediately dorsal to the DRN (Derer, 1981). In cats, a [³H]-labelled proline injection in DRN and raphe centralis superior labelled supraependymal surfaces of all ventricles by means of anterograde transport (Pierce et al., 1976).

Studies on the rat lateral ventricles indicate that serotonergic fibres do not penetrate the ependyma, but instead enter the ventricles from their rostral poles. These fibres travel along fibres that travel through the median forebrain bundle and turn dorsocaudally between the CP and corpus callosum. Also, they do not form synaptic contact with ependymal cells. They are not found between ependyma and subependyma, but only in the lateral ventricles (Dinopoulos et al., 1995).

Neurotransmitters

DRN neurons utilize several other neurotransmitters (Fig. 3). This chapter will list such transmitters, but not those, which are located in afferent fibres to the DRN and synthesized elsewhere.

Serotonin

Serotonin is the main neurotransmitter of the DRN and the first one to be demonstrated there (Dahlstrom and Fuxe, 1964). The serotonergic DRN neurons and their projections have been described in more detail in other parts of this chapter.

Dopamine

Dopamine was one of the first transmitters, to be demonstrated in DRN neurons, first with histo-fluorescence methods (Lindvall and Bjorklund, 1974; Ochi and Shimizu, 1978) and later with antibodies against tyrosine hydroxylase (TH) and dopamine- β -hydroxylase (D β H) (Nagatsu et al., 1979). These dopaminergic neurons are located preferentially in ventromedial parts. They mainly target the NA and lateral septum, and to a lesser extent medial prefrontal cortex. In addition, very few fibres project to CP (Stratford and Wirtshafter, 1990).

GABA

GABAergic neurons were first demonstrated in the DRN by radioautographic tracing and GABA-uptake (Belin et al., 1979). The observation was supported by immunohistochemistry with an antibody against the GABA-synthesizing enzyme γ -aminobutyric acid decarboxylase, or GAD (Mugnaini and Oertel, 1985) and the GABA-degrading enzyme GABA-transaminase, or GABA-T (Nagai et al., 1983). The GABAergic synapse with serotonergic DRN neurons (Wang et al., 1992). They are markedly smaller than most serotonergic neurons and fire spikes characterized by short width and high frequency (Allers and Sharp, 2003).

Peptide transmitters

Immunohistochemical stainings have shown that the DRN harbours neuropeptide Y (NPY) containing neurons, most of which are medium-sized, fusiform and bipolar (de Quidt and Emson, 1986). In situ-hybridization has demonstrated the presence of NPY mRNA in the DRN (Pau et al., 1998).

Substance P has been shown to colocalize with serotonin in the DRN in at least rat (Chan-Palay et al., 1978; Hokfelt et al., 1978), cat (Lovick and Hunt, 1983; Arvidsson et al., 1994) and human (Baker et al., 1990, 1991). Substance P also colocalizes with serotonin in ascending projections, but such fibres have not been shown to arise from the DRN (Otake, 2005). However, in another study no colocalization was seen in ascending fibres (Rupniak and Kramer, 1999).

Low levels of prepro-*galanin* mRNA are present in DRN neurons (Cortes et al., 1990), yet galanin itself has been detected with immunohistochemistry only after colchicine treatment (Skofitsch and Jacobowitz, 1985). Galanin colocalizes with serotonin in the DRN. In fact, it has been reported that a large proportion of serotonergic DRN neurons also contain galanin (Melander et al., 1986). Galanin is also present in serotonergic fibres in one of the target areas of the DRN, the cortex (Skofitsch and Jacobowitz, 1985), but it has not been confirmed that these projections arise in the DRN.

Enkephalin (ENK)-containing neurons were first reported in the dorsal and lateral parts of rat DRN, just adjacent to the periventricular grey matter (Hokfelt et al., 1977; Uhl et al., 1979). Immunohistochemical studies showed that ENK is present throughout the cat DRN in neurons of variable morphology (Moss et al., 1980, 1981). However, serotonergic double-labelled neurons were predominantly small and round and located at the midline, dorsal to the medial longitudinal fasciculus (Glazer et al., 1981).

CRF immunoreactivity has been demonstrated in DRN neurons after colchicine treatment (Commons et al., 2003). CRF-immunoreactive neurons were mainly clustered in the dorsomedial subregion, especially in the middle DRN. Scattered neurons were seen in the lateral wings, while they

were largely absent from the ventromedial DRN and the most caudal part of the DRN. Most (~96%) of CRF-immunoreactive neurons in the dorsomedial DRN were serotonergic, as defined by immunoreactivity for TPH. Anterograde tracing (PHA-L) indicated that neurons in the middle portion of the dorsomedial DRN mainly target the CeA, the dorsal hypothalamic area and the bed nucleus of the stria terminalis (Commons et al., 2003).

In additional *vasoactive intestinal polypeptide* (VIP) has been demonstrated in neurons of both rat and mouse DRN (Sims et al., 1980) and *cholecystokinin* (CKK)-containing neurons in the rat DRN have been shown to innervate the PVN of the thalamus (Bhatnagar et al., 2000; Otake, 2005).

Glutamate

Phosphate-activated glutaminase (PAG) has been demonstrated in TH-, D β H- or phenylethanolamine-*N*-methyltransferase (PNMT)-immunoreactive neurons, suggesting that glutamate is formed from glutamine in serotonergic and catecholaminergic neurons of the DRN (Kaneko et al., 1990).

Nitric oxide

The presence of nitric oxide (NO) in DRN was first demonstrated by immunohistochemistry against the NO synthesis reaction product citrulline (Pasqualotto et al., 1991) and against argininosuccinate synthetase which turns citrulline into argininosuccinate (Nakamura et al., 1991). Subsequently, the presence of NO in both serotonergic and non-serotonergic DRN neurons was demonstrated by colocalization of serotonin-immunoreactivity with immunoreactivity for NO synthase (NOS) (Dun et al., 1994; Rodrigo et al., 1994) or with NADPH diaphorase activity (Johnson and Ma, 1993; Wotherspoon et al., 1994). The NO-synthesizing neurons are predominantly clustered in medioventral and mediodorsal parts of DRN (Wang et al., 1995). In the medial subnuclei, between 23 and 38% of serotonergic neurons appear to synthesize NO, whereas 60–77% of NO-synthesizing neurons are serotonergic. In the lateral subregions, NADPH diaphorase activity is present, but its activity does not overlap

with serotonergic neurons (Wotherspoon et al., 1994).

Transient presence of additional transmitters

At least two additional neurotransmitters have been reported in the developing, but not adult, DRN. *Histamine* is present in neurons of rat and mouse DRN during embryonic development, but disappears before birth, as demonstrated by the presence of histamine-immunoreactivity and histidine decarboxylase (the histamine-synthesizing enzyme) mRNA (Auvinen and Panula, 1988; Nissinen and Panula, 1995; Nissinen et al., 1995; Karlstedt et al., 2001). Recent studies have shown that the gastrointestinal peptide *secretin* is also present in the DRN during mouse embryonic development (Lossi et al., 2004).

Plasticity

During the last few decades the traditional view of the adult brain as a static network of cells and fibres has given way for increased understanding of the importance of plasticity in the CNS. Neuroplasticity is now seen as an indispensable trait, which allows the CNS to adjust to its environment, as a result of experience or following injury, by undergoing adaptive changes at several structural and functional levels. It encompasses, for instance, the outgrowth or shrinkage of dendrites and axons, as well as neurochemical changes at the synapse. In addition, neurogenesis has fairly recently been added to the list.

Among the vast number of functions attributed to serotonin, is regulation of many forms of neural plasticity. It has been proposed that the serotonergic system of the DRN and other raphe nuclei, with its plastic properties, is a key player in the brain's integration with the rest of the body and the environment (Azmitia, 1999). The proposal is in line with, but extends beyond, previous concepts, and states that the serotonergic system has a unique and wide homeostatic role, which involves feedback regulation by a variety of neuronal and non-neuronal factors in the body,

including neurotransmitters, glucocorticoids, steroids and oxygen.

It is by now well established that serotonin regulates sprouting, synaptic plasticity and neurogenesis and thereby seems to be deeply involved in the regulation of most forms of neuroplasticity.

Sprouting

Serotonergic fibres are plastic in the adult brain. Lesioning studies have shown that serotonergic axons are able to regenerate very fast, as first shown in the spinal cord (Nobin et al., 1973) and hypothalamus (Frankfurt and Azmitia, 1984). Serotonergic neurons also affect fibre plasticity in their target cells and areas, and can have both promoting and inhibiting effects, although most studies have demonstrated that serotonin stimulates sprouting. For instance, serotonin triggered growth cone retraction in the chick dorsal root ganglion (Igarashi et al., 1995) and inhibited neurite outgrowth in goldfish retina (Lima et al., 1994). In addition, PC12 cells developed neurites in the presence of serotonin (Severin and Kondratyev, 1988) and serotonin enhanced neurite outgrowth in thalamic mouse (Lotto et al., 1999) and rat (Lieske et al., 1999) neurons in vitro.

Synaptogenesis

Serotonin has a stimulating effect on synaptogenesis. Serotonin depletion by treatment with the TPH inhibitor *p*-chlorophenylalanine (PCPA) leads to synapse loss in adult rat cortex, hippocampus and hypothalamus (Chen et al., 1994; Azmitia et al., 1995), as well as in early postnatal rat hippocampus (Mazer et al., 1997). TPH inhibition also leads to learning deficits in rat (Mazer et al., 1997).

Neurogenesis

The traditional view of the mammalian nervous system as being entirely postmitotic, has only recently been challenged, and changed, by the discovery of neurogenesis in the adult mammalian brain (Eriksson et al., 1998). Already in the 1960s, ³H-autoradiography studies identified new

neurons in the rat brain (Altman and Das, 1965) but, for a long time, the finding did not receive the attention it would have deserved. Almost two decades later, neurogenesis was demonstrated convincingly in the canary forebrain (Goldman and Nottebohm, 1983), and during the 1990s it became accepted that in humans, primates and rodents, proliferation and neurogenesis take place in two areas of the adult brain: the subgranular layer of the dentate gyrus of the hippocampus and the subventricular zone (Momba et al., 2000). Both areas are targeted by fibres from the raphe nuclei and a multitude of evidence points to serotonin as a key player in the regulation of neurogenesis. For instance, lesions to the raphe nuclei lead to decreased cell proliferation in the hippocampus, but the effect can be counteracted by a raphe transplant (Brezun and Daszuta, 2000) and selective serotonin reuptake inhibitors (SSRI) increase neurogenesis (Malberg, 2004).

A putative link between serotonin and neurogenesis is brain-derived neurotrophic factor (BDNF). BDNF promotes cell survival, synaptic plasticity and neurogenesis, and acts in concert with serotonin: BDNF enhances the survival and growth of serotonergic neurons (Mamounas et al., 1995) whereas serotonin stimulates BDNF expression. (Jankowsky and Patterson, 1999; Mattson et al., 2004). BDNF is a target for the cyclic adenosine monophosphate (cAMP) responsive element (CREB) of the cAMP-signalling cascade and, consequently, cAMP and CREB have also been implicated in serotonin-mediated neurogenesis (D'Sa and Duman, 2002; Manji et al., 2003).

Major depression and Alzheimer's disease

Via its ascending projections, the DRN plays an important role in the regulation of many physiological functions. These include learning, memory and affect. Consequently, a dysfunctional serotonergic system has been implicated in disorders related to these functions, for instance major depression and AD. We shall focus on these two diseases, including a suggested link between major depression and AD.

Major depression

Major depression is one of the most common psychiatric diseases. It has an incidence of about 4% and a lifetime prevalence of 12–20% in Europe (Alonso et al., 2004; Paykel et al., 2005) and thus, a deeper understanding of its mechanisms is of high clinical importance. Dysfunction of the serotonergic system has been linked to depression, and although a dysfunctional serotonin system alone cannot explain the full pathophysiology, it is considered a key factor in depression.

The first implication of a connection between serotonin and depression was made in the early 1960s, when the first antidepressant, iproniazid, was found to inhibit the enzyme MAO B, which degrades serotonin and other monoamines. Subsequently, the search for drugs, which would selectively enhance the transmission of a single monoamine, led to the development of SSRI. SSRI enhance serotonergic signalling by inhibiting the reuptake of the transmitter from the synaptic cleft and constitute the most successful antidepressants today.

As a major source of serotonergic input to the forebrain, the DRN has naturally received much attention in depression research. Recent evidence includes a postmortem study, which found a 31% decrease in overall neuron number in the DRN of depressed patients with a mean age of 50 years (Baumann et al., 2002). On the other hand, another study found no decrease in DRN neuron number and pathology in elderly people who had suffered from depression (Hendricksen et al., 2004). This may reflect differences in the aetiology between depression among middle-aged and elderly. In addition, TPH immunoreactivity and mRNA levels in the DRN are higher in depressed suicide victims than in controls (Boldrini et al., 2005; Bach-Mizrahi et al., 2006). One should not, however, focus only on the DRN itself, but rather on the entire serotonergic system, including the target areas of the fibres emerging from the raphe nuclei.

Neuroplasticity in depression

It has been proposed that major depression could be caused, at least partly, by disturbed neurogenesis

(Duman et al., 2000a; Jacobs et al., 2000). For instance, imaging studies revealed shrinkage of the hippocampus in the brain of patients with stress-related mood disorders such as major depression (Sheline et al., 1996, 2003).

The hypothesis is also supported by animal studies, which show that antidepressants, such as SSRI, can increase neurogenesis in the hippocampus (Malberg et al., 2000; Duman et al., 2001a, b). Electroconvulsive therapy (ECT), which is widely used as antidepressant therapy, has a similar effect (Madsen et al., 2000). In addition, it has been shown that hippocampal neurogenesis is a prerequisite of antidepressant-induced behavioural changes, because X-irradiation-induced disruption of hippocampal neurogenesis prevented the behavioural antidepressant effects of the SSRI imipramine and fluoxetine (Santarelli et al., 2003).

BDNF is considered to be an important link between antidepressant therapy and neurogenesis. As already mentioned, BDNF acts in concert with serotonin to promote cell survival, synaptic plasticity and neurogenesis. Both human and animal studies provide an increasing amount of evidence of the interplay between serotonin and BDNF that has accumulated during the last few years, as well as evidence linking BDNF function directly to depression: BDNF protein was increased in the hippocampus of depressed patients who were treated with antidepressants (Chen et al., 2001) and was decreased in suicide victims (Karege et al., 2005). Chronic stress decreased the expression of BDNF mRNA (Smith et al., 1995) and BDNF protein levels (Xu et al., 2002, 2006) in the hippocampus of rats. In animal models of depression, BDNF itself has been shown to have an antidepressant effect (Siuciak et al., 1997; Shirayama et al., 2002) and antidepressant therapy, including ECT, can antagonize stress-induced decreases in BDNF levels in normal rats (Nibuya et al., 1995). The scheme is further supported by studies showing that neurogenesis in the hippocampus and dentate gyrus volume are reduced in BDNF knock-out mice (Lee et al., 2002). As a conclusion, the effect of increased intrasynaptic serotonin levels as a result of, for instance, the inhibition of serotonin reuptake by SSRI could stimulate BDNF expression which, in turn,

promotes cell survival, synaptic plasticity and neurogenesis (Malberg, 2004; Mattson et al., 2004). Serotonergic neurons are also affected by BDNF, thereby creating a positive plasticity-promoting feedback loop.

The Val66Met (G194A) single nucleotide polymorphism (SNP) may partially explain the variation in the size of the hippocampal formation (Szeszko et al., 2005). This might be linked to the finding that this SNP is associated with major depression, although it is probably not itself directly responsible for an increased susceptibility for major depression (Schumacher et al., 2005). This SNP has also been associated with anxiety-related behaviour, just as the C281A SNP in the BDNF promoter. Heterozygous carriers of the C281A polymorphism seemed to be less anxious than persons, who did not carry the polymorphism at all. The Val66Met polymorphism, on the other hand, was most abundant in persons with a history of both anxiety and major depression (Jiang et al., 2005). This is agreement with an animal study, where transgenic mice carrying two alleles of the Val66Met SNP displayed increased anxiety-related behaviour, and did not respond to SSRI treatment (Chen et al., 2006). Finally, two independent lines of a conditional forebrain BDNF homozygote (−/−) knock-out mice have revealed sex differences in depression-related behaviour: female BDNF knock-outs displayed increased depression-related behaviour, whereas no similar effect was observed in males, which only displayed increase locomotor activity (Monteggia et al., 2007). Interestingly, female BDNF knock-outs showed increased anxiety-like behaviour whereas males were normal. Thus, these findings implicate forebrain BDNF in a depression-type found in women who are hyporesponsive to environmental stimuli (Monteggia et al., 2007).

Duman and coworkers have suggested that the entire signalling cascade cAMP — CREB — BDNF should be considered (D'Sa and Duman, 2002; Duman and Monteggia, 2006). This signal transduction cascade is upregulated after long-term antidepressant treatment, presumably in order to stimulate neuroplasticity, including neurogenesis (D'Sa and Duman, 2002; Hashimoto et al., 2004). Of note, in contrast to the hippocampus,

stimulation of this pathway in NA or amygdala produces a prodepressant effect (Newton et al., 2002; Wallace et al., 2004). Activation of CREB, BDNF expression and subsequent neurogenesis is a fairly slow process, which is consistent with the notion that antidepressant treatment induces long-term cellular changes. Consequently, this might explain why antidepressants are effective only after prolonged treatment (Duman et al., 2000b; Fricker et al., 2005).

Serotonin receptors

Many studies on the mechanisms behind depression have focused on serotonin receptors. The 5HT_{1A} receptor has received particular attention. Patients suffering from major depression show reduced hippocampal 5HT_{1A} receptor mRNA levels and receptor binding (Cheetham et al., 1990; Lowther et al., 1997; Lopez-Figueroa et al., 2004) and a PET imaging study found that 5HT_{1A} receptor binding was reduced by more than 40% in the raphe nuclei in untreated depressed patients as compared to healthy controls. A decrease in binding was also observed in, e.g. the mediotemporal cortex, but nowhere was it as large as in the raphe (Drevets et al., 1999). Furthermore, depressed patients were twice as likely as controls to carry two copies of a polymorphism in the 5HT_{1A} receptor promoter (C1019G). Among suicide victims, homozygotes, with respect to the same allele, were four times as common as among controls (Lemondé et al., 2003).

5HT_{1A} receptor densities correlate with hypothalamic-pituitary-adrenal (HPA) axis activity and responsiveness in the rat (Burnet et al., 1992), and 5HT_{1A} gene expression is under tonic inhibition of corticosterone (Burnet et al., 1992; Chalmers et al., 1994). 5HT_{1A} receptor-mediated activation of the serotonergic system has been shown to be involved in antidepressant-induced neurogenesis in adult mice: treatment with the 5HT_{1A} receptor agonist 6OH-DPAT increased cell proliferation in the hippocampus and was antidepressant in normal mice, but the effect was absent in knock-out mice, which lacked the 5-HT_{1A} receptor (Santarelli et al., 2003).

In reserpine-treated rats, which is a model of depression, 5-HT_{1A} receptor immunoreactivity was decreased in the pyramidal cell layer of the hippocampus (Iritani et al., 2006). Interestingly, in BDNF homozygote (–/–) knock-out mice, the 5HT_{1A} receptor function, but not the number of the 5-HT_{1A} receptors was decreased (Hensler et al., 2007). Of note, this was only studied in male mice which do not show clear anxiety- and depression-related behaviour yet as observed in female BDNF (–/–) knock-out mice. The anxiolytic effects of 5HT_{1A} receptor agonists (Griebel, 1995) and increased anxiety-like behaviour of 5HT_{1A} knock-out mice further support the notion that the receptor activation mediates anxiolytic behaviour as well (Ramboz et al., 1998).

In addition, both 5HT_{1A} and 5HT_{1B} receptors regulate cell proliferation in the subgranular layer of the hippocampus (Banasz et al., 2004). A recent study by Svenningsson et al. (2006) also points to the involvement of 5HT_{1B} receptors. The levels of the protein p11, which recruits 5HT_{1B} receptors to the cell membrane (Svenningsson and Greengard, 2007), was shown to be decreased in an animal model of depression and in human postmortem brains from depressed patients. In addition, p11 can be increased by antidepressants and electroconvulsive treatment in the normal rodent brain and p11 knock-out mice display a depression-like phenotype despite having increased levels of serotonin. Further, the distribution of p11 is similar to that of 5HT_{1B} receptors and is present, e.g. in the DRN and some of its target areas. Interestingly, a treatment-induced (imipramine) rise in p11 did not occur in the DRN, where 5HT_{1B} receptors function as autoreceptors, but only in the forebrain (e.g. cortex) where it is associated with 5HT_{1B} heteroreceptors (Svenningsson et al., 2006).

Other serotonergic receptors have also been linked to depression, albeit to a lesser extent. For instance decreased 5HT_{2A} receptor binding has been demonstrated in depressed suicide victims (Cheetham et al., 1988; Rosel et al., 1998, 2000, 2004) and in patients with major depression (Mintun et al., 2004). However, a recent study found no differences in 5HT_{2A} mRNA expression between patients with a history of major

depression and controls (Lopez-Figueroa et al., 2004). In rats, 5HT_{2A} and 5HT_{2C} receptors in the subgranular layer of the dentate gyrus and the subventricular zone, respectively, have been implicated in depression: 5HT_{2A} receptor blockade leads to a decrease in cell proliferation in the subgranular layer of the rat hippocampus, and 5HT_{2A} receptor loss correlates with anxiety (Chen et al., 2000). Activation of 5HT_{2C} produces an increase in proliferation in the subventricular zone (Banast et al., 2004) and it has been suggested that a decrease in 5HT_{2C} and increase in 5HT₃ receptors may fasten the onset of antidepressants (Dremencov et al., 2006).

Studies by Graeff et al. (1993) suggested the importance of a balance between 5HT_{1A} and 5HT_{2A} receptor activation in the regulation of conditioned fear. When serotonin was injected into the amygdala and the periaqueductal grey, conditioned fear was enhanced and inhibited, respectively. The authors have suggested that serotonergic DRN projections to the amygdala mediate anxiogenic effects by activating the 5HT_{2A} receptors whereas MRN projections to the hippocampus, as well as DRN projections to the periventricular and aqueductal grey matter, suppress the expression of fear (flight/fight reactions) via activation of the 5HT_{1A} receptors (Graeff et al., 1993, 1996).

Tryptophan hydroxylase 2

A loss of function C1473G SNP in one of the human serotonin-synthesizing enzymes, TPH2 has been linked to major depression. In mice the same mutation led to a 50–70% reduction in the rate of serotonin synthesis in cortex and striatum and a 40% reduction in serotonin levels in homozygous carriers. When expressed in cell cultures, the mutant allele caused a 55% decrease in serotonin levels (Zhang et al., 2004). The same laboratory has identified another SNP (Arg441His) in elderly (>60 years) unipolar depression patients. Approximately 10% carried the mutant allele, against only 1.3% in a control group. All of the three control subjects carrying the allele, suffered from either generalized anxiety symptoms or mild

depression, and a high ratio of the depressed patients seemed to suffer from a severe form of the disease. In cells expressing the mutant form of TPH2, the serotonin levels were 80% lower than in controls (Zhang et al., 2005a). However, several other groups have failed to find the allele altogether despite very large sample sizes (see Blakely, 2005 and other comments in the same journal issue; Delorme et al., 2006). The discrepancy remains to be elucidated, but raises the possibility that the Arg441His mutation is related to a rare, severe form of late-onset depression (Zhang et al., 2005b). No TPH2 knock-out mouse is yet available.

Serotonin transporter

Serotonin transporter polymorphisms seem to underlie at least a few per cent of affective disorders (Heils et al., 1997). Of two common alleles, a short one (s) leads to decreased SERT mRNA expression and serotonin reuptake in vitro as compared to the long one (l). In healthy humans the s allele has been associated with anxiety-related features and in an fMRI study, individuals carrying the s allele displayed a stronger amygdala response to fearful stimuli than those homozygous for the l allele (Lesch et al., 1996; Hariri et al., 2002). In addition, a longitudinal study by Caspi et al. (2003) found that homozygous carriers of s allele were more likely to develop depression as a result of childhood maltreatment, than those carrying the l allele only. Thus, it seems that the SERT s allele leads to disturbed serotonergic signalling, which magnifies the impact of adverse life events on the brain (Lesch et al., 1996; Caspi et al., 2003).

In parallel to the SERT polymorphisms in human, mutant mice for the SERT have been generated. SERT homozygote (–/–) knock-out mice displayed increased anxiety-like behaviour (Holmes et al., 2003) as well as depression-like behaviour (Carroll et al., 2007). Also the antidepressant effect of the selective serotonin reuptake inhibitor fluoxetine was abolished in these mice (Holmes et al., 2002). Double mutant mice with SERT–/– and BDNF+/- showed an increased anxiety-like behaviour when compared with both the single knock-out mutants (Ren-Patterson et al.,

2005). Moreover, they had about a 30% reduction in dendrites of hippocampal neurons in comparison to the wild-type mice (Ren-Patterson et al., 2005). Interestingly, transgenic mice overexpressing the SERT also showed an increased anxiety-like behaviour (Jennings et al., 2006). Thus, changes in 5-HT transmission, either due to increased or decreased extracellular 5-HT levels, will eventually have effective consequences.

CRF and the HPA axis

CRF activates the HPA axis and mediates the acute stress response. After a stressful event, CRF levels decrease and HPA axis function returns to normal. However, if the presence of a stressor is prolonged, chronic stress maintains high CRF secretion, which has been implicated in anxiety and depression (Leonard, 2005). The effect of CRF is mediated by two (CRF1 and 2) receptors which seem to have opposite effects: mice that are deficient in CRF2 receptors show an increase in anxiety and stress responses (Kishimoto et al., 2000), whereas CRF1 deficiency has the opposite effect in rodents (Smith et al., 1995).

CRF is linked to serotonin and the DRN system in two ways. Firstly, the CRF-mediated stress response and glucocorticoids have major effects on serotonin 5HT1A and 5HT2A receptors. Both receptors subtypes have been implicated in depression (see the above section 'Serotonin receptors') and it has been suggested that a deficiency in the activity of either receptor type following HPA axis activation, may lead to anxiety-related pathology, e.g. by sensitization and desensitization of 5HT1A and 5HT2A receptors, respectively (Leonard, 2005). Secondly, some serotonergic neurons in the dorsomedial portion of the DRN are CRF-immunoreactive (Commons et al., 2003), raising the possibility that the DRN neurons may modify the HPA axis directly via CRF output, and not only via serotonin. The target areas of the dorsomedial DRN include amygdala and hypothalamus, which also contain CRF2 receptors. In addition, collateral DRN efferents have been shown to target both areas simultaneously (Petrov et al., 1994). At least in macaque monkeys, DRN neurons

also innervate the bed nucleus of stria terminalis (Freedman and Shi, 2001), through which amygdaloid CRF neurons project to several targets, including the DRN (Gray, 1993; Leonard, 2005).

It has been proposed that GABAergic DRN neurons are involved in the CRF-mediated regulation of serotonergic neurons in the DRN. CRF could activate GABAergic neurons by binding to CRF1 receptors. As a consequence, the inhibitory tone on serotonergic neurons would increase. CRF2 receptor activation would inhibit GABAergic neurons and thus have the opposite result (Valentino and Commons, 2005).

It has also been proposed that substance P, acting via NK1 receptors in the dorsal DRN, could selectively activate CRF/serotonin-immunoreactive neurons in the same area, while inhibiting more ventrally located serotonergic DRN neurons (Valentino and Commons, 2005). Since the CRF/serotonin-neurons target the amygdala (Commons et al., 2003), substance P could activate this pathway, while inhibiting other efferent DRN projections. Indeed, NK1 receptor activation has similar anxiogenic effects as CRF administration to the amygdala, whereas NK1 antagonists have anxiolytic effects (Gray and Bingaman, 1996). Administration of NK1 receptor antagonists has little effect on neural discharge in the DRN, which indicates that substance P is not released in large quantities under basal conditions (Haddjeri and Blier, 2001; Valentino et al., 2003). Instead, it could be activated under certain physiological conditions, possibly leading to increased anxiogenic CRF-output to the amygdala, as suggested by Valentino and Commons (2005). Interestingly, the projections from the DRN seem to have a facilitatory effect on HPA axis activity, whereas median raphe efferents mediate the opposite effect (Lowry, 2002).

Alzheimer's disease

Neuroplasticity in the ageing brain

Several studies show that neuronal plasticity is decreased in normal ageing. For instance, young rats recover faster and more completely from ischaemic damage (Yager et al., 2006). In addition,

the expression of the genes encoding for plasticity-related molecules, such as BDNF and its high-affinity receptor *trkB*, is decreased in normal ageing (Croll et al., 1998). The same is true for the gene encoding the NR1 subunit of the NMDA receptor, involved in LTP (Eckles-Smith et al., 2000).

Interestingly, such changes seem to be absent in the brains of mice subjected to dietary restriction. Dietary restriction, i.e. access to sufficient, but not excessive amounts of food, can extend the lifespan of rodents and keep them, including their brain, healthier in old age (McCay et al., 1935; Sohal and Weindruch, 1996). Gene-expression measurements have shown that BDNF gene expression is 1.8-fold higher in 30-month old dietary restricted mice as compared to mice, with access to food *ad libitum*, and thus similar to the levels in normal young mice (Lee et al., 2000; Duan et al., 2001; Prolla and Mattson, 2001). At least one additional plasticity-related protein, neuroserpin, is also upregulated (1.9-fold) in the same dietary restricted mouse model (Prolla and Mattson, 2001). In addition, the age-related decrease in the expression of the NR1 NMDA receptor subunit can be prevented by dietary restriction (Eckles-Smith et al., 2000).

Exercise also leads to a marked increase in BDNF transcription and increase in neurogenesis (Neeper et al., 1996; Russo-Neustadt et al., 1999, 2000). This could have important implications in the recovery from ischaemia through exercise, as suggested by rat experiments (Kim et al., 2005) but also in the appreciation of physical activity to stimulate plasticity in the healthy ageing brain. An enriched environment also seems to stimulate neuroplasticity (Mattson et al., 2004). For instance, age-related decrease in synaptic density can be counteracted by maintaining rats in an enriched environment (Saito et al., 1994) and rodents with access to many objects to play with when growing up, display increased neurogenesis (Kempermann et al., 1997). In order to test the combined effect of physical exercise and an enriched environment, Mahncke and coworkers designed a training programme for the elderly, aimed at maintaining brain plasticity. It contained demanding sensory, cognitive and motor activities, which older adults could engage in without

supervision. The programme resulted in improvements in memory. Such brain plasticity based programmes could prove to be useful on a broader scale, as suggested by Mahncke et al. (2006).

As a conclusion, neuroplasticity undergoes age-related decrease, which can be slowed down or halted with dietary restriction, sensory stimulation and exercise. Optimally, a combination of all three components in the daily life of older adults could postpone brain ageing and improve the quality of life.

Neuroplasticity in Alzheimer's disease

DRN neurons decrease in number in AD (Curcio and Kemper, 1984; Yamamoto and Hirano, 1985; Aletrino et al., 1992). Chen et al. (2000), who recorded a 41% neuron loss in the DRN of AD patients, found no correlation between DRN pathology and cognitive decline or non-cognitive behavioural change in these patients. As suggested by the authors, a possible explanation is that the remaining serotonergic neurons are able to take over the function of the lost neurons. Nevertheless, AD is strongly associated with neurodegeneration in the DRN. Neuron loss has been reported to be most severe in the caudal DRN (Zweig et al., 1988). From here, neurons innervate at least the lateral septum and the hippocampus, which is the most affected structure in AD in term of neurodegeneration and amyloid plaques formation.

Another indication for increased neurodegeneration and decreased neuroplasticity in the brain of AD patients comes from the observation that BDNF mRNA and BDNF protein levels are decreased in different brain structures including the hippocampus (Phillips et al., 1991; Narisawa-Saito et al., 1996; Connor et al., 1997). More in detail, neuritis surrounding senile plaques have high BDNF levels further underlining the involvement of BDNF in neuronal degeneration and/or compensatory mechanisms (Murer et al., 1999). Similar findings have been observed in several transgenic mouse models of AD (Burbach et al., 2004; Wolf et al., 2006; Wu et al., 2006). It can be suggested that the reduction of BDNF in brain

structures such as the hippocampus may also contribute to the observed cognitive deficits in AD as conditional forebrain-restricted BDNF homozygote knock-out mice showed profound impairments in spatial learning, which is hippocampus-dependent (Gorski et al., 2003).

The Val66Met (G194A) SNP within the BDNF gene, which modifies neuronal BDNF secretion, has been associated with major depression as mentioned before, but it is also linked to memory impairments (Bath and Lee, 2006) and the development of sporadic AD (Matsushita et al., 2005). A C270T SNP within the BDNF gene has also been linked to the onset of AD (Kunugi et al., 2001). Of note, there are ethnic differences in whether such SNPs increase the risk of AD. Since BDNF is related to major depression as well, it has been suggested that BDNF could be a bridge between AD and major depression, explaining both the depressive symptoms in AD and the cognitive impairment in major depression (Tsai, 2003).

Serotonergic transmission

Serotonergic transmission is impaired in AD. Studies on human postmortem material from AD patients have consistently shown that the serotonergic neuron density in the DRN of AD patients is reduced by approximately 40–50% (Zweig et al., 1988; Chen et al., 2000; Hendricksen et al., 2004). The disease has also been associated with decreased serotonin receptor binding in several brain areas (Cross et al., 1984). Studies on neocortical biopsy samples showed that serotonin and 5-hydroxyindoleacetic acid concentrations, serotonin uptake and serotonin release were decreased (Palmer et al., 1987). In addition, SERT activity was decreased in the DRN and the hippocampus (Tejani-Butt et al., 1995).

The HPA axis

The hippocampus provides an inhibitory input to the HPA axis. An impaired hippocampal function would implicate an affected HPA axis function. Hippocampal damage is one of the major

hallmarks of AD and it has been observed that HPA axis function is disturbed in AD. This leads to elevated cortisol levels in AD patients (Davis et al., 1986; Masugi et al., 1989). High cortisol levels in AD patients are inversely related to cognitive performance and associated with hippocampal dysfunction (Pomara et al., 2003), and excess cortisol administration in healthy humans is associated with impaired memory (Newcomer et al., 1999; de Quervain et al., 2000). Thus, the effects of high cortisol seem to resemble some of AD-related pathology, which makes the HPA axis an interesting study-object in AD research as well with possible implications for affective disorders (see also below).

Depression as a risk factor for Alzheimer's disease and vice versa

There is ample evidence to suggest that a history of depression constitutes a risk factor for developing AD later in life. Although some studies have yielded contrasting results, most studies have found a higher incidence of AD among patients who have suffered from depression. A meta-analysis involving 20 case-control or case studies, recently confirmed that such a correlation exists (Ownby et al., 2006). It has been suggested that depression may, in some cases, constitute an early sign for AD. However, this seems not to be the case, as the interval between first diagnoses of depression and AD is positively related to the risk for developing AD (Green et al., 2003; Ownby et al., 2006).

In a study on AD patients, a history of depression also correlated with an increased formation of the classical pathological hallmarks of AD, namely amyloid plaques and neurofibrillary tangles, in the hippocampus (Rapp et al., 2006). Of these patients, those who at the time of AD diagnosis were suffering from depression had an even higher number of plaques and tangles. The same study showed that a history of depression also correlated with the rate of cognitive decline, indicating that the neurodegenerative process was accelerated (Rapp et al., 2006). A history of depression has also been associated with loss of hippocampal volume in the elderly (Sheline et al.,

2003). A possible underlying mechanism is that depression leads to a downregulation of normal hippocampal neurogenesis, thereby resulting in neuron loss over time. However, at least two human postmortem studies have failed to detect hippocampal neuron loss, but instead attribute the volume loss to pathological synaptic reorganization (Muller et al., 2001) or increased packing density of neurons and glia (Stockmeier et al., 2004). The neurogenesis hypothesis is supported by studies using a transgenic mouse model for AD (APP_{K670NM671NL}), which overexpresses a mutant form of the APP gene. Such mice display decreased hippocampal cell proliferation (Haughey et al., 2002; Dong et al., 2004); an effect, which is further amplified by isolation stress. Interestingly, isolation stress also accelerated amyloid plaque deposition in the hippocampus (Dong et al., 2004).

Some studies suggest that depression leads to increased DRN neuron loss in AD patients. Among AD patients, those who had suffered from depression had significantly fewer neurons in DRN (and LC) than non-depressed AD patients (Zweig et al., 1988). In a more recent study on elderly subjects, no depression-associated differences in the number of serotonergic neurons were found in the DRN of AD patients, regardless of whether they had also suffered from AD (Hendricksen et al., 2004). This discrepancy is somewhat puzzling but it is possible that the number of non-serotonergic, but not serotonergic, neurons in the DRN is decreased in depression. Furthermore, the patient groups used by Hendricksen and coworkers were not age-matched and thus, age was used as a covariate. Curiously, before age-correction, AD patients with depression showed a similar 34% decrease in neuron number as compared to AD patients without depression. This must, however, be seen as a consequence of the depressive patients' older age.

Not only is depression a risk factor for AD, but the incidence of depression is higher among patients with AD than age-matched controls (Olin et al., 2002). One possible explanation could be disturbances in regional cerebral blood flow (rCBF) as a consequence of AD-related changes in the serotonergic system. Such changes could include loss of serotonergic DRN neurons, which

would lead to decreased serotonergic innervation of DRN target areas of the forebrain. Interestingly, serotonergic fibres surround major cerebral arteries and pial microvessels (Chan-Palay, 1976) and play a potent vasoconstrictor role (Parsons, 1991) while depression strongly correlates with abnormal rCBF (Soares and Mann, 1997; Drevets, 2001; Mayberg, 2003). The role of rCBF in depression is supported by studies, which show that some antidepressant treatments normalize blood flow in specific areas in depressive patients (Drevets, 2000; Mayberg, 2003; Zobel et al., 2005). This suggests that there may be a link between AD-related depression and decreased cerebral blood flow due to serotonergic dysfunction. Should this be the case, it could have important implications on the treatment of depressive AD patients.

The pathological hallmarks of AD may also have an impact on depression. A link between tangles and depression has been suggested in a study with a transgenic mouse model, carrying a mutant form of human tau (R406W), which causes accumulation of neurofibrillary tangles, in which tau protein is a main component (Egashira et al., 2005). The transgenic mice displayed increased depression-like behaviour, which was counteracted by the SSRI fluvoxamine. The drug also elevated the abnormally low serotonin levels in the brain of the transgenic mouse, suggesting that tangle formation impairs the functioning of the serotonergic system (Egashira et al., 2005).

The involvement of HPA axis dysfunction in both depression and AD, as reviewed above, may be a key to elucidating the causal relationship between the two diseases. Rat experiments have indicated that glucocorticoids aggravate excitotoxicity-induced tangle formation in the hippocampus (Elliott et al., 1993) and affect A β -processing (Budasz et al., 1999). Furthermore, administration of glucocorticoids to triple transgenic mice with APP_{KM670/671NL}PS1_{M146V}tau_{301L} mutations resulted in increased hippocampal amyloid deposition and tau accumulation (Green et al., 2006). In addition, hippocampal-dependent learning in the water escape task was impaired in these mice (Nelson et al., 2007). Interestingly, the same study demonstrated that

this effect and the increased amyloid accumulation were attenuated after 5 months of treatment with the SSRI paroxetine. Thus, hippocampal damage due to plaque and tangle formation may disrupt the negative feedback to the HPA axis, which augments cortisol levels, creating a vicious circle where plaque and tau formation is increased further (Pomara et al., 2003). This effect might be most relevant during the early stages of the disease, as suggested by a study by Swanwick et al. (1998), where HPA axis dysfunction did not become worse over time and with increased cognitive decline in AD patients (Swanwick et al., 1998). Thus, HPA axis dysfunction may be an early trigger for subsequent development of AD.

Taken together, depression seems to be a risk factor for the development of AD, and AD seems to be linked to a higher incidence of depression. The prime purpose of an effective treatment of patients with depression is, of course, to alleviate their suffering from that disease, but given the link between these two diseases, the role of depression as a risk factor should further motivate the fast and effective treatment of depressive patients, in order to decrease their risk of developing AD. Concurrently, depression among AD patients should be diagnosed and viewed upon as a separate, treatable disease.

Acknowledgements

Some parts of this chapter have been published in Michelsen et al. (2007) with permission from Elsevier. KAM and JP are supported by European Union Framework 6 Integrated Project NEW-MOOD Grant LSHM-CT-2004-503474 and KAM by grants from Helsingin Sanomain 100-vuotisäätiö, Alfred Kordelinin yleinen edistys- ja sivistysrahasto, Orionin tutkimussäätiö and K. Albin Johanssons stiftelse.

References

- Abrams, J.K., Johnson, P.L., Hollis, J.H. and Lowry, C.A. (2004) Anatomic and functional topography of the dorsal raphe nucleus. *Ann. N.Y. Acad. Sci.*, 1018: 46–57.
- Aghajanian, G.K. and Gallager, D.W. (1975) Raphe origin of serotonergic nerves terminating in the cerebral ventricles. *Brain Res.*, 88: 221–231.
- Aletrino, M.A., Vogels, O.J., Van Domburg, P.H. and ten Donkelaar, H.J. (1992) Cell loss in the nucleus raphe dorsalis in Alzheimer's disease. *Neurobiol. Aging*, 13: 461–468.
- Alheid, G.F. and Heimer, L. (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience*, 27: 1–39.
- Allers, K.A. and Sharp, T. (2003) Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience*, 122: 193–204.
- Alonso, J., Angermeyer, M.C., Bernert, S., Bruffaerts, R., Brugha, T.S., Bryson, H., de Girolamo, G., Graaf, R., Demyttenaere, K., Gasquet, I., Haro, J.M., Katz, S.J., Kessler, R.C., Kovess, V., Lepine, J.P., Ormel, J., Polidori, G., Russo, L.J., Vilagut, G., Almansa, J., Arbabzadeh-Bouchez, S., Autonell, J., Bernal, M., Buist-Bouwman, M.A., Codony, M., Domingo-Salvany, A., Ferrer, M., Joo, S.S., Martinez-Alonso, M., Matschinger, H., Mazzi, F., Morgan, Z., Morosini, P., Palacin, C., Romera, B., Taub, N. and Vollebergh, W.A. (2004) Prevalence of mental disorders in Europe: results from the European study of the epidemiology of mental disorders (ESEMeD) project. *Acta. Psychiatr. Scand. Suppl.*, 21–27.
- Altman, J. and Das, G.D. (1965) Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.*, 124: 319–335.
- Anden, N.E., Dahlstrom, A., Fuxe, K. and Larsson, K. (1965) Mapping out of catecholamine and 5-hydroxytryptamine neurons innervating the telencephalon and diencephalon. *Life Sci.*, 4: 1275–1279.
- Araneda, S., Bobillier, P., Buda, M. and Pujol, J.F. (1980a) Retrograde axonal transport following injection of [3H]serotonin in the olfactory bulb. I. Biochemical study. *Brain Res.*, 196: 405–415.
- Araneda, S., Gamrani, H., Font, C., Calas, A., Pujol, J.F. and Bobillier, P. (1980b) Retrograde axonal transport following injection of [3H]-serotonin into the olfactory bulb. II. Radioautographic study. *Brain Res.*, 196: 417–427.
- Arvidsson, U., Cullheim, S., Ulfhake, B., Luppi, P.H., Kitahama, K., Jouvet, M. and Hokfelt, T. (1994) Quantitative and qualitative aspects on the distribution of 5-HT and its coexistence with substance P and TRH in cat ventral medullary neurons. *J. Chem. Neuroanat.*, 7: 3–12.
- Auvinen, S. and Panula, P. (1988) Development of histamine-immunoreactive neurons in the rat brain. *J. Comp. Neurol.*, 276: 289–303.
- Azmitia, E.C. (1999) Serotonin neurons, neuroplasticity, and homeostasis of neural tissue. *Neuropsychopharmacology*, 21: 33S–45S.
- Azmitia, E.C., Rubinstein, V.J., Strafacci, J.A., Rios, J.C. and Whitaker-Azmitia, P.M. (1995) 5-HT_{1A} agonist and dexamethasone reversal of para-chloroamphetamine induced loss

- of MAP-2 and synaptophysin immunoreactivity in adult rat brain. *Brain Res.*, 677: 181–192.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179: 641–667.
- Bach-Mizrachi, H., Underwood, M.D., Kassir, S.A., Bakalian, M.J., Sibille, E., Tamir, H., Mann, J.J. and Arango, V. (2006) Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: major depression and suicide. *Neuropsychopharmacology*, 31: 814–824.
- Baker, K.G., Halliday, G.M., Hornung, J.P., Geffen, L.B., Cotton, R.G. and Tork, I. (1991) Distribution, morphology and number of monoamine-synthesizing and substance P-containing neurons in the human dorsal raphe nucleus. *Neuroscience*, 42: 757–775.
- Baker, K.G., Halliday, G.M. and Tork, I. (1990) Cytoarchitecture of the human dorsal raphe nucleus. *J. Comp. Neurol.*, 301: 147–161.
- Banasr, M., Hery, M., Printemps, R. and Daszuta, A. (2004) Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the sub-ventricular zone. *Neuropsychopharmacology*, 29: 450–460.
- Bath, K.G. and Lee, F.S. (2006) Variant BDNF (Val66Met) impact on brain structure and function. *Cogn. Affect. Behav. Neurosci.*, 6: 79–85.
- Baumann, B., Biellau, H., Krell, D., Agelink, M.W., Diekmann, S., Wurthmann, C., Trubner, K., Bernstein, H.G., Danos, P. and Bogerts, B. (2002) Circumscribed numerical deficit of dorsal raphe neurons in mood disorders. *Psychol. Med.*, 32: 93–103.
- Belin, M.F., Aguera, M., Tappaz, M., McRae-Degueurce, A., Bobillier, P. and Pujol, J.F. (1979) GABA-accumulating neurons in the nucleus raphe dorsalis and periaqueductal gray in the rat: a biochemical and radioautographic study. *Brain Res.*, 170: 279–297.
- Bhatnagar, S., Viau, V., Chu, A., Soriano, L., Meijer, O.C. and Dallman, M.F. (2000) A cholecystokinin-mediated pathway to the paraventricular thalamus is recruited in chronically stressed rats and regulates hypothalamic-pituitary-adrenal function. *J. Neurosci.*, 20: 5564–5573.
- Blakely, R.D. (2005) Overview: a rare opportunity or just one less reason to be depressed. *Neuron*, 48: 701–702.
- Bobillier, P., Seguin, S., Petitjean, F., Salvat, D., Touret, M. and Jouvett, M. (1976) The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Res.*, 113: 449–486.
- Boldrini, M., Underwood, M.D., Mann, J.J. and Arango, V. (2005) More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. *Brain Res.*, 1041: 19–28.
- Braak, H. (1970) Über die Kerngebiete de menschlichen Hirnstammes. II Die Raphekerne. *Z. Zellforsch. Mikrosk. Anat.*, 107: 123–141.
- Brezun, J.M. and Daszuta, A. (2000) Serotonergic reinnervation reverses lesion-induced decreases in PSA-NCAM labeling and proliferation of hippocampal cells in adult rats. *Hippocampus*, 10: 37–46.
- Brown, P. and Molliver, M.E. (2000) Dual serotonin (5-HT) projections to the nucleus accumbens core and shell: relation of the 5-HT transporter to amphetamine-induced neurotoxicity. *J. Neurosci.*, 20: 1952–1963.
- Budas, G., Coughlan, C.M., Seckl, J.R. and Breen, K.C. (1999) The effect of corticosteroids on amyloid beta precursor protein/amyloid precursor-like protein expression and processing in vivo. *Neurosci. Lett.*, 276: 61–64.
- Bunney, B.S. and Aghajanian, G.K. (1976) The precise localization of nigral afferents in the rat as determined by a retrograde tracing technique. *Brain Res.*, 117: 423–435.
- Burbach, G.J., Hellweg, R., Haas, C.A., Del Turco, D., Deicke, U., Abramowski, D., Jucker, M., Staufenbiel, M. and Deller, T. (2004) Induction of brain-derived neurotrophic factor in plaque-associated glial cells of aged APP23 transgenic mice. *J. Neurosci.*, 24: 2421–2430.
- Burnet, P.W., Mefford, I.N., Smith, C.C., Gold, P.W. and Sternberg, E.M. (1992) Hippocampal 8-[3H]hydroxy-2-(di-*n*-propylamino) tetralin binding site densities, serotonin receptor (5-HT1A) messenger ribonucleic acid abundance, and serotonin levels parallel the activity of the hypothalamopituitary-adrenal axis in rat. *J. Neurochem.*, 59: 1062–1070.
- Carroll, J.C., Boyce-Rustay, J.M., Millstein, R., Yang, R., Wiedholz, L.M., Murphy, D.L. and Holmes, A. (2007) Effects of mild early life stress on abnormal emotion-related behaviors in 5-HTT knockout mice. *Behav. Genet.*, 37: 214–222.
- Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. and Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, 301: 386–389.
- Chalmers, D.T., Lopez, J.F., Vazquez, D.M., Akil, H. and Watson, S.J. (1994) Regulation of hippocampal 5-HT1A receptor gene expression by dexamethasone. *Neuropsychopharmacology*, 10: 215–222.
- Chan-Palay, V. (1976) Serotonin axons in the supra- and subependymal plexuses and in the leptomeninges; their roles in local alterations of cerebrospinal fluid and vasomotor activity. *Brain Res.*, 102: 103–130.
- Chan-Palay, V., Jonsson, G. and Palay, S.L. (1978) Serotonin and substance P coexist in neurons of the rat's central nervous system. *Proc. Natl. Acad. Sci. U.S.A.*, 75: 1582–1586.
- Cheetham, S.C., Crompton, M.R., Katona, C.L. and Horton, R.W. (1988) Brain 5-HT₂ receptor binding sites in depressed suicide victims. *Brain Res.*, 443: 272–280.
- Cheetham, S.C., Crompton, M.R., Katona, C.L. and Horton, R.W. (1990) Brain 5-HT₁ binding sites in depressed suicides. *Psychopharmacology (Berl.)*, 102: 544–548.
- Chen, B., Dowlatsahi, D., MacQueen, G.M., Wang, J.F. and Young, L.T. (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry*, 50: 260–265.
- Chen, C.P.L., Eastwood, S.L., Hope, T., McDonald, B., Francis, P.T. and Esiri, M.M. (2000) Immunocytochemical

- study of the dorsal and median raphe nuclei in patients with Alzheimer's disease prospectively assessed for behavioural changes. *Neuropathol. Appl. Neurobiol.*, 26: 347–355.
- Chen, L., Hamaguchi, K., Ogawa, M., Hamada, S. and Okada, N. (1994) PCPA reduces both monoaminergic afferents and nonmonoaminergic synapses in the cerebral cortex. *Neurosci. Res.*, 19: 111–115.
- Chen, Z.Y., Jing, D., Bath, K.G., Ieraci, A., Khan, T., Siao, C.J., Herrera, D.G., Toth, M., Yang, C., McEwen, B.S., Hempstead, B.L. and Lee, F.S. (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, 314: 140–143.
- Commons, K.G., Connolly, K.R. and Valentino, R.J. (2003) A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology*, 28: 206–215.
- Connor, B., Young, D., Yan, Q., Faull, R.L., Synek, B. and Dragunow, M. (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res. Mol. Brain Res.*, 49: 71–81.
- Conrad, L.C., Leonard, C.M. and Pfaff, D.W. (1974) Connections of the median and dorsal raphe nuclei in the rat: an autoradiographic and degeneration study. *J. Comp. Neurol.*, 156: 179–205.
- Cortes, R., Ceccatelli, S., Schalling, M. and Hokfelt, T. (1990) Differential effects of intracerebroventricular colchicine administration on the expression of mRNAs for neuropeptides and neurotransmitter enzymes, with special emphasis on galanin: an in situ hybridization study. *Synapse*, 6: 369–391.
- Croll, S.D., Ip, N.Y., Lindsay, R.M. and Wiegand, S.J. (1998) Expression of BDNF and trkB as a function of age and cognitive performance. *Brain Res.*, 812: 200–208.
- Cross, A.J., Crow, T.J., Ferrier, I.N., Johnson, J.A., Bloom, S.R. and Corsellis, J.A. (1984) Serotonin receptor changes in dementia of the Alzheimer type. *J. Neurochem.*, 43: 1574–1581.
- Curcio, C.A. and Kemper, T. (1984) Nucleus raphe dorsalis in dementia of the Alzheimer type: neurofibrillary changes and neuronal packing density. *J. Neuropathol. Exp. Neurol.*, 43: 359–368.
- D'Sa, C. and Duman, R.S. (2002) Antidepressants and neuroplasticity. *Bipolar Disord.*, 4: 183–194.
- Dahlstrom, A. and Fuxe, K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.*, 62(Suppl.): p. 55.
- Davis, K.L., Davis, B.M., Greenwald, B.S., Mohs, R.C., Mathe, A.A., Johns, C.A. and Horvath, T.B. (1986) Cortisol and Alzheimer's disease, I: basal studies. *Am. J. Psychiatry*, 143: 300–305.
- de Quervain, D.J., Roozendaal, B., Nitsch, R.M., McGaugh, J.L. and Hock, C. (2000) Acute cortisone administration impairs retrieval of long-term declarative memory in humans. *Nat. Neurosci.*, 3: 313–314.
- de Quidt, M.E. and Emson, P.C. (1986) Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system-II. Immunohistochemical analysis. *Neuroscience*, 18: 545–618.
- Delorme, R., Durand, C.M., Betancur, C., Wagner, M., Ruhrmann, S., Grabe, H.J., Nygren, G., Gillberg, C., Leboyer, M., Bourgeron, T., Courtet, P., Jollant, F., Buresi, C., Aubry, J.M., Baud, P., Bondolfi, G., Bertschy, G., Perroud, N. and Malafosse, A. (2006) No human tryptophan hydroxylase-2 gene R441H mutation in a large cohort of psychiatric patients and control subjects. *Biol. Psychiatry*, 60: 202–203.
- Derer, P. (1981) The supraependymal fibres (SEF) of the mouse brain as visualized by the Golgi method. *J. Physiol. (Paris)*, 77: 211–218.
- Descarries, L., Watkins, K.C., Garcia, S. and Beaudet, A. (1982) The serotonin neurons in nucleus raphe dorsalis of adult rat: a light and electron microscope radioautographic study. *J. Comp. Neurol.*, 207: 239–254.
- DeVito, J.L., Anderson, M.E. and Walsh, K.E. (1980) A horseradish peroxidase study of afferent connections of the globus pallidus in *Macaca mulatta*. *Exp. Brain Res.*, 38: 65–73.
- Dinopoulos, A., Dori, I. and Parnavelas, J.G. (1995) Serotonergic innervation of the lateral geniculate nucleus of the rat during postnatal development: a light and electron microscopic immunocytochemical analysis. *J. Comp. Neurol.*, 363: 532–544.
- Dong, H., Goico, B., Martin, M., Csernansky, C.A., Bertchume, A. and Csernansky, J.G. (2004) Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience*, 127: 601–609.
- Dremencov, E., Weizmann, Y., Kinor, N., Gispan-Herman, I. and Yadid, G. (2006) Modulation of dopamine transmission by 5HT2C and 5HT3 receptors: a role in the antidepressant response. *Curr. Drug Targets*, 7: 165–175.
- Drevets, W.C. (2000) Neuroimaging studies of mood disorders. *Biol. Psychiatry*, 48: 813–829.
- Drevets, W.C. (2001) Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr. Opin. Neurobiol.*, 11: 240–249.
- Drevets, W.C., Frank, E., Price, J.C., Kupfer, D.J., Holt, D., Greer, P.J., Huang, Y., Gautier, C. and Mathis, C. (1999) PET imaging of serotonin 1A receptor binding in depression. *Biol. Psychiatry*, 46: 1375–1387.
- Duan, W., Lee, J., Guo, Z. and Mattson, M.P. (2001) Dietary restriction stimulates BDNF production in the brain and thereby protects neurons against excitotoxic injury. *J. Mol. Neurosci.*, 16: 1–12.
- Duman, R.S., Malberg, J. and Nakagawa, S. (2001a) Regulation of adult neurogenesis by psychotropic drugs and stress. *J. Pharmacol. Exp. Ther.*, 299: 401–407.
- Duman, R.S., Nakagawa, S. and Malberg, J. (2001b) Regulation of adult neurogenesis by antidepressant treatment. *Neuropsychopharmacology*, 25: 836–844.
- Duman, R.S., Malberg, J., Nakagawa, S. and D'Sa, C. (2000a) Neuronal plasticity and survival in mood disorders. *Biol. Psychiatry*, 48: 732–739.

- Duman, R.S., Malberg, J., Nakagawa, S. and D'Sa, C. (2000b) Neuronal plasticity and survival in mood disorders. *Biol. Psychiatry*, 48: 732–739.
- Duman, R.S. and Monteggia, L.M. (2006) A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry*, 59: 1116–1127.
- Dun, N.J., Dun, S.L. and Forstermann, U. (1994) Nitric oxide synthase immunoreactivity in rat pontine medullary neurons. *Neuroscience*, 59: 429–445.
- Eckles-Smith, K., Clayton, D., Bickford, P. and Browning, M.D. (2000) Caloric restriction prevents age-related deficits in LTP and in NMDA receptor expression. *Brain Res. Mol. Brain Res.*, 78: 154–162.
- Egashira, N., Iwasaki, K., Takashima, A., Watanabe, T., Kawabe, H., Matsuda, T., Mishima, K., Chidori, S., Nishimura, R. and Fujiwara, M. (2005) Altered depression-related behavior and neurochemical changes in serotonergic neurons in mutant R406W human tau transgenic mice. *Brain Res.*, 1059: 7–12.
- Elliott, E.M., Mattson, M.P., Vanderklish, P., Lynch, G., Chang, I. and Sapolsky, R.M. (1993) Corticosterone exacerbates kainate-induced alterations in hippocampal tau immunoreactivity and spectrin proteolysis in vivo. *J. Neurochem.*, 61: 57–67.
- Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A. and Gage, F.H. (1998) Neurogenesis in the adult human hippocampus. *Nat. Med.*, 4: 1313–1317.
- Falck, B., Hillarp, N.A., Thieme, G. and Torp, A. (1962) Fluorescence of catecholamines and related compounds with formaldehyde. *J. Histochem. Cytochem.*, 10: 348–354.
- Felten, D.L. and Cummings, J.P. (1979) The raphe nuclei of the rabbit brain stem. *J. Comp. Neurol.*, 187: 199–243.
- Fibiger, H.C. and Miller, J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience*, 2: 975–987.
- Frankfurt, M. and Azmitia, E. (1984) Regeneration of serotonergic fibers in the rat hypothalamus following unilateral 5,7-dihydroxytryptamine injection. *Brain Res.*, 298: 273–282.
- Freedman, L.J. and Shi, C. (2001) Monoaminergic innervation of the macaque extended amygdala. *Neuroscience*, 104: 1067–1084.
- Fricker, A.D., Rios, C., Devi, L.A. and Gomes, I. (2005) Serotonin receptor activation leads to neurite outgrowth and neuronal survival. *Brain Res. Mol. Brain Res.*, 138: 228–235.
- Geyer, M.A., Puerto, A., Dawsey, W.J., Knapp, S., Bullard, W.P. and Mandell, A.J. (1976) Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.*, 106: 241–256.
- Glazer, E.J., Steinbusch, H., Verhofstad, A. and Basbaum, A.I. (1981) Serotonin neurons in nucleus raphe dorsalis and paragigantocellularis of the cat contain enkephalin. *J. Physiol. (Paris)*, 77: 241–245.
- Goldman, S.A. and Nottebohm, F. (1983) Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc. Natl. Acad. Sci. U.S.A.*, 80: 2390–2394.
- Gorski, J.A., Balogh, S.A., Wehner, J.M. and Jones, K.R. (2003) Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. *Neuroscience*, 121: 341–354.
- Graeff, F.G., Guimaraes, F.S., De Andrade, T.G. and Deakin, J.F. (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacol. Biochem. Behav.*, 54: 129–141.
- Graeff, F.G., Silveira, M.C., Nogueira, R.L., Audi, E.A. and Oliveira, R.M. (1993) Role of the amygdala and periaqueductal gray in anxiety and panic. *Behav. Brain Res.*, 58: 123–131.
- Gray, T.S. (1993) Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. *Ann. N.Y. Acad. Sci.*, 697: 53–60.
- Gray, T.S. and Bingaman, E.W. (1996) The amygdala: corticotropin-releasing factor, steroids, and stress. *Crit. Rev. Neurobiol.*, 10: 155–168.
- Green, K.N., Billings, L.M., Roozendaal, B., McGaugh, J.L. and LaFerla, F.M. (2006) Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J. Neurosci.*, 26: 9047–9056.
- Green, R.C., Cupples, L.A., Kurz, A., Auerbach, S., Go, R., Sadovnick, D., Duara, R., Kukull, W.A., Chui, H., Edeki, T., Griffith, P.A., Friedland, R.P., Bachman, D. and Farrer, L. (2003) Depression as a risk factor for Alzheimer disease: the MIRAGE study. *Arch. Neurol.*, 60: 753–759.
- Griebel, G. (1995) 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol. Ther.*, 65: 319–395.
- Grove, E.A. (1988) Neural associations of the substantia innominata in the rat: afferent connections. *J. Comp. Neurol.*, 277: 315–346.
- Haddjeri, N. and Blier, P. (2001) Sustained blockade of neurokinin-1 receptors enhances serotonin neurotransmission. *Biol. Psychiatry*, 50: 191–199.
- Halaris, A.E., Jones, B.E. and Moore, R.Y. (1976) Axonal transport in serotonin neurons of the midbrain raphe. *Brain Res.*, 107: 555–574.
- Halberstadt, A.L. and Balaban, C.D. (2006) Serotonergic and nonserotonergic neurons in the dorsal raphe nucleus send collateralized projections to both the vestibular nuclei and the central amygdaloid nucleus. *Neuroscience*, 140: 1067–1077.
- Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F. and Weinberger, D.R. (2002) Serotonin transporter genetic variation and the response of the human amygdala. *Science*, 297: 400–403.
- Hashimoto, K., Shimizu, E. and Iyo, M. (2004) Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res. Brain Res. Rev.*, 45: 104–114.
- Haughey, N.J., Nath, A., Chan, S.L., Borchard, A.C., Rao, M.S. and Mattson, M.P. (2002) Disruption of neurogenesis by amyloid beta-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. *J. Neurochem.*, 83: 1509–1524.

- Hay-Schmidt, A., Vrang, N., Larsen, P.J. and Mikkelsen, J.D. (2003) Projections from the raphe nuclei to the supra-chiasmatic nucleus of the rat. *J. Chem. Neuroanat.*, 25: 293–310.
- Heils, A., Mossner, R. and Lesch, K.P. (1997) The human serotonin transporter gene polymorphism: basic research and clinical implications. *J. Neural. Transm.*, 104: 1005–1014.
- Hendricksen, M., Thomas, A.J., Ferrier, I.N., Ince, P. and O'Brien, J.T. (2004) Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *Am. J. Psychiatry*, 161: 1096–1102.
- Hensler, J.G., Advani, T. and Monteggia, L.M. (2007) Regulation of serotonin-1A receptor function in inducible brain-derived neurotrophic factor knockout mice after administration of corticosterone. *Biol. Psychiatry*, 62: 521–529.
- Hokfelt, T., Ljungdahl, A., Steinbusch, H., Verhofstad, A., Nilsson, G., Brodin, E., Pernow, B. and Goldstein, M. (1978) Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neuroscience*, 3: 517–538.
- Hokfelt, T., Ljungdahl, A., Terenius, L., Elde, R. and Nilsson, G. (1977) Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. *Proc. Natl. Acad. Sci. U.S.A.*, 74: 3081–3085.
- Holmes, A., Yang, R.J., Lesch, K.P., Crawley, J.N. and Murphy, D.L. (2003) Mice lacking the serotonin transporter exhibit 5-HT(1A) receptor-mediated abnormalities in tests for anxiety-like behavior. *Neuropsychopharmacology*, 28: 2077–2088.
- Holmes, A., Yang, R.J., Murphy, D.L. and Crawley, J.N. (2002) Evaluation of antidepressant-related behavioral responses in mice lacking the serotonin transporter. *Neuropsychopharmacology*, 27: 914–923.
- Igarashi, M., Li, W.W., Sudo, Y. and Fishman, M.C. (1995) Ligand-induced growth cone collapse: amplification and blockade by variant GAP-43 peptides. *J. Neurosci.*, 15: 5660–5667.
- Imai, H., Steindler, D.A. and Kitai, S.T. (1986) The organization of divergent axonal projections from the midbrain raphe nuclei in the rat. *J. Comp. Neurol.*, 243: 363–380.
- Iritani, S., Tohgi, M., Arai, T. and Ikeda, K. (2006) Immunohistochemical study of the serotonergic neuronal system in an animal model of the mood disorder. *Exp. Neurol.*, 201: 60–65.
- Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.*, 72: 165–229.
- Jacobs, B.L., Praag, H. and Gage, F.H. (2000) Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol. Psychiatry*, 5: 262–269.
- Jankowsky, J.L. and Patterson, P.H. (1999) Cytokine and growth factor involvement in long-term potentiation. *Mol. Cell. Neurosci.*, 14: 273–286.
- Jennings, K.A., Loder, M.K., Sheward, W.J., Pei, Q., Deacon, R.M., Benson, M.A., Olverman, H.J., Hastie, N.D., Harmar, A.J., Shen, S. and Sharp, T. (2006) Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. *J. Neurosci.*, 26: 8955–8964.
- Jiang, X., Xu, K., Hoberman, J., Tian, F., Marko, A.J., Waheed, J.F., Harris, C.R., Marini, A.M., Enoch, M.A. and Lipsky, R.H. (2005) BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology*, 30: 1353–1361.
- Johnson, M.D. and Ma, P.M. (1993) Localization of NADPH diaphorase activity in monoaminergic neurons of the rat brain. *J. Comp. Neurol.*, 332: 391–406.
- Kaneko, T., Akiyama, H., Nagatsu, I. and Mizuno, N. (1990) Immunohistochemical demonstration of glutaminase in catecholaminergic and serotonergic neurons of rat brain. *Brain Res.*, 507: 151–154.
- Karege, F., Vaudan, G., Schwald, M., Perroud, N. and La Harpe, R. (2005) Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res. Mol. Brain Res.*, 136: 29–37.
- Karlstedt, K., Nissinen, M., Michelsen, K.A. and Panula, P. (2001) Multiple sites of l-histidine decarboxylase expression in mouse suggest novel developmental functions for histamine. *Dev. Dyn.*, 221: 81–91.
- Kellar, K.J., Brown, P.A., Madrid, J., Bernstein, M., Vernikos-Danellis, J. and Mehler, W.R. (1977) Origins of serotonin innervation of forebrain structures. *Exp. Neurol.*, 56: 52–62.
- Kempermann, G., Kuhn, H.G. and Gage, F.H. (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature*, 386: 493–495.
- Kim, M.W., Bang, M.S., Han, T.R., Ko, Y.J., Yoon, B.W., Kim, J.H., Kang, L.M., Lee, K.M. and Kim, M.H. (2005) Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic rat brain. *Brain Res.*, 1052: 16–21.
- Kirifides, M.L., Simpson, K.L., Lin, R.C. and Waterhouse, B.D. (2001) Topographic organization and neurochemical identity of dorsal raphe neurons that project to the trigeminal somatosensory pathway in the rat. *J. Comp. Neurol.*, 435: 325–340.
- Kishimoto, T., Radulovic, J., Radulovic, M., Lin, C.R., Schrick, C., Hooshmand, F., Hermanson, O., Rosenfeld, M.G. and Spiess, J. (2000) Deletion of *crhr2* reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat. Genet.*, 24: 415–419.
- Kohler, C., Chan-Palay, V. and Steinbusch, H. (1982) The distribution and origin of serotonin-containing fibers in the septal area: a combined immunohistochemical and fluorescent retrograde tracing study in the rat. *J. Comp. Neurol.*, 209: 91–111.
- Kohler, C. and Steinbusch, H. (1982) Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. *Neuroscience*, 7: 951–975.

- Kosofsky, B.E. and Molliver, M.E. (1987) The serotonergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse*, 1: 153–168.
- Kunugi, H., Ueki, A., Otsuka, M., Isse, K., Hirasawa, H., Kato, N., Nabika, T., Kobayashi, S. and Nanko, S. (2001) A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. *Mol. Psychiatry*, 6: 83–86.
- Lee, J., Duan, W., Long, J.M., Ingram, D.K. and Mattson, M.P. (2000) Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats. *J. Mol. Neurosci.*, 15: 99–108.
- Lee, J., Duan, W. and Mattson, M.P. (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.*, 82: 1367–1375.
- Leger, L. and Wiklund, L. (1982) Distribution and numbers of indoleamine cell bodies in the cat brainstem determined with Falck–Hillarp fluorescence histochemistry. *Brain Res. Bull.*, 9: 245–251.
- Lemondé, S., Turecki, G., Bakish, D., Du, L., Hrdina, P.D., Bown, C.D., Sequeira, A., Kushwaha, N., Morris, S.J., Basak, A., Ou, X.M. and Albert, P.R. (2003) Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J. Neurosci.*, 23: 8788–8799.
- Leonard, B.E. (2005) The HPA and immune axes in stress: the involvement of the serotonergic system. *Eur. Psychiatry*, 20(Suppl. 3): S302–S306.
- Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H. and Murphy, D.L. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274: 1527–1531.
- Lieske, V., Bennett-Clarke, C.A. and Rhoades, R.W. (1999) Effects of serotonin on neurite outgrowth from thalamic neurons in vitro. *Neuroscience*, 90: 967–974.
- Lima, L., Matus, P. and Urbina, M. (1994) Serotonin inhibits outgrowth of goldfish retina and impairs the trophic effect of taurine. *J. Neurosci. Res.*, 38: 444–450.
- Lindvall, O. and Bjorklund, A. (1974) The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta. Physiol. Scand. Suppl.*, 412: 1–48.
- Lopez-Figueroa, A.L., Norton, C.S., Lopez-Figueroa, M.O., Armellini-Dodel, D., Burke, S., Akil, H., Lopez, J.F. and Watson, S.J. (2004) Serotonin 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. *Biol. Psychiatry*, 55: 225–233.
- Lorez, H.P. and Richards, J.G. (1982) Supra-ependymal serotonergic nerves in mammalian brain: morphological, pharmacological and functional studies. *Brain Res. Bull.*, 9: 727–741.
- Lossi, L., Bottarelli, L., Candusso, M.E., Leiter, A.B., Rindi, G. and Merighi, A. (2004) Transient expression of secretin in serotonergic neurons of mouse brain during development. *Eur. J. Neurosci.*, 20: 3259–3269.
- Lotto, B., Upton, L., Price, D.J. and Gaspar, P. (1999) Serotonin receptor activation enhances neurite outgrowth of thalamic neurones in rodents. *Neurosci. Lett.*, 269: 87–90.
- Lovick, T.A. and Hunt, S.P. (1983) Substance P-immunoreactive and serotonin-containing neurones in the ventral brainstem of the cat. *Neurosci. Lett.*, 36: 223–228.
- Lowry, C.A. (2002) Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. *J. Neuroendocrinol.*, 14: 911–923.
- Lowther, S., De Paermentier, F., Cheetham, S.C., Crompton, M.R., Katona, C.L. and Horton, R.W. (1997) 5-HT_{1A} receptor binding sites in post-mortem brain samples from depressed suicides and controls. *J. Affect Disord.*, 42: 199–207.
- Madsen, T.M., Treschow, A., Bengzon, J., Bolwig, T.G., Lindvall, O. and Tingstrom, A. (2000) Increased neurogenesis in a model of electroconvulsive therapy. *Biol. Psychiatry*, 47: 1043–1049.
- Mahncke, H.W., Bronstone, A. and Merzenich, M.M. (2006) Brain plasticity and functional losses in the aged: scientific bases for a novel intervention. *Prog. Brain Res.*, 157: 81–109.
- Maier, S.F. and Watkins, L.R. (2005) Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neurosci. Biobehav. Rev.*, 29: 829–841.
- Malberg, J.E. (2004) Implications of adult hippocampal neurogenesis in antidepressant action. *J. Psychiatry Neurosci.*, 29: 196–205.
- Malberg, J.E., Eisch, A.J., Nestler, E.J. and Duman, R.S. (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.*, 20: 9104–9110.
- Mamounas, L.A., Blue, M.E., Siuciak, J.A. and Altar, C.A. (1995) Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. *J. Neurosci.*, 15: 7929–7939.
- Mamounas, L.A. and Molliver, M.E. (1988) Evidence for dual serotonergic projections to neocortex: axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin *p*-chloroamphetamine (PCA). *Exp. Neurol.*, 102: 23–36.
- Mamounas, L.A., Mullen, C.A., O'Hearn, E. and Molliver, M.E. (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. *J. Comp. Neurol.*, 314: 558–586.
- Manji, H.K., Gottesman, I.I. and Gould, T.D. (2003) Signal transduction and genes-to-behaviors pathways in psychiatric diseases. *Sci. STKE*, 2003: p. e49.
- Masugi, F., Ogihara, T., Sakaguchi, K., Otsuka, A., Tsuchiya, Y., Morimoto, S., Kumahara, Y., Saeki, S. and Nishide, M. (1989) High plasma levels of cortisol in patients with senile dementia of the Alzheimer's type. *Methods Find. Exp. Clin. Pharmacol.*, 11: 707–710.
- Matsushita, S., Arai, H., Matsui, T., Yuzuriha, T., Urakami, K., Masaki, T. and Higuchi, S. (2005) Brain-derived

- neurotrophic factor gene polymorphisms and Alzheimer's disease. *J. Neural. Transm.*, 112: 703–711.
- Mattson, M.P., Maudsley, S. and Martin, B. (2004) BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.*, 27: 589–594.
- Mayberg, H.S. (2003) Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *Br. Med. Bull.*, 65: 193–207.
- Mazer, C., Muneyirci, J., Taheny, K., Raio, N., Borella, A. and Whitaker-Azmitia, P. (1997) Serotonin depletion during synaptogenesis leads to decreased synaptic density and learning deficits in the adult rat: a possible model of neurodevelopmental disorders with cognitive deficits. *Brain Res.*, 760: 68–73.
- McCay, C.M., Crowell, M.F. and Maynard, L.A. (1935) The effect of retarded growth upon the length of the lifespan and upon the ultimate body size. *J. Nutr.*, 10: 63–79.
- McQuade, R. and Sharp, T. (1997) Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *J. Neurochem.*, 69: 791–796.
- Melander, T., Hökfelt, T., Rokaeus, A., Cuello, A.C., Oertel, W.H., Verhofstad, A. and Goldstein, M. (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *J. Neurosci.*, 6: 3640–3654.
- Meyer-Bernstein, E.L. and Morin, L.P. (1996) Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J. Neurosci.*, 16: 2097–2111.
- Michelsen, K.A., Schmitz, C. and Steinbusch, H.W. (2007) The dorsal raphe nucleus: from silver stainings to a role in depression. *Brain Res. Rev.*, 55: 329–342.
- Miller, J.J., Richardson, T.L., Fibiger, H.C. and McLennan, H. (1975) Anatomical and electrophysiological identification of a projection from the mesencephalic raphe to the caudate-putamen in the rat. *Brain Res.*, 97: 133–136.
- Mintun, M.A., Sheline, Y.I., Moerlein, S.M., Vlassenko, A.G., Huang, Y. and Snyder, A.Z. (2004) Decreased hippocampal 5-HT_{2A} receptor binding in major depressive disorder: in vivo measurement with [¹⁸F]altanserin positron emission tomography. *Biol. Psychiatry*, 55: 217–224.
- Momma, S., Johansson, C.B. and Frisen, J. (2000) Get to know your stem cells. *Curr. Opin. Neurobiol.*, 10: 45–49.
- Monteggia, L.M., Luikart, B., Barrot, M., Theobald, D., Malkovska, I., Nef, S., Parada, L.F. and Nestler, E.J. (2007) Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol. Psychiatry*, 61: 187–197.
- Morin, L.P. and Meyer-Bernstein, E.L. (1999) The ascending serotonergic system in the hamster: comparison with projections of the dorsal and median raphe nuclei. *Neuroscience*, 91: 81–105.
- Moss, M.S., Glazer, E.J. and Basbaum, A.I. (1980) Enkephalin neurons in the raphe dorsalis and periaqueductal grey of the cat: a comparison with substance P. *Anat. Rec.*, 196: 131A.
- Moss, M.S., Glazer, E.J. and Basbaum, A.I. (1981) Enkephalin-immunoreactive perikarya in the cat raphe dorsalis. *Neurosci. Lett.*, 21: 33–37.
- Mugnaini, E. and Oertel, W.H. (1985) An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In: Björklund A. and Hökfelt T. (Eds.), *Handbook of Chemical Neuroanatomy*, Vol. 4. GABA and Neuropeptides in the CNS. Part I Elsevier Science Publishers, B.V., Amsterdam, pp. 436–608.
- Muller, M.B., Lucassen, P.J., Yassouridis, A., Hoogendijk, W.J., Holsboer, F. and Swaab, D.F. (2001) Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus. *Eur. J. Neurosci.*, 14: 1603–1612.
- Mulligan, K.A. and Tork, I. (1988) Serotonergic innervation of the cat cerebral cortex. *J. Comp. Neurol.*, 270: 86–110.
- Murer, M.G., Boissiere, F., Yan, Q., Hunot, S., Villares, J., Faucheux, B., Agid, Y., Hirsch, E. and Raisman-Vozari, R. (1999) An immunohistochemical study of the distribution of brain-derived neurotrophic factor in the adult human brain, with particular reference to Alzheimer's disease. *Neuroscience*, 88: 1015–1032.
- Nagai, T., McGeer, P.L. and McGeer, E.G. (1983) Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. *J. Comp. Neurol.*, 218: 220–238.
- Nagatsu, I., Inagaki, S., Kondo, Y., Karasawa, N. and Nagatsu, T. (1979) Immunofluorescent studies on the localization of tyrosine hydroxylase and dopamine- α -hydroxylase in the mes-, di-, and telencephalon of the rat using unperfused fresh frozen sections. *Acta Histochem. Cytochem.*, 12: 20–37.
- Nakahama, H., Shima, K., Yamamoto, M. and Aya, K. (1981) Regularity of the spontaneous discharge of neurons in the nucleus raphe dorsalis of the cat. *Neurosci. Lett.*, 23: 161–165.
- Nakamura, H., Saheki, T., Ichiki, H., Nakata, K. and Nakagawa, S. (1991) Immunocytochemical localization of argininosuccinate synthetase in the rat brain. *J. Comp. Neurol.*, 312: 652–679.
- Narisawa-Saito, M., Wakabayashi, K., Tsuji, S., Takahashi, H. and Nawa, H. (1996) Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer's disease. *Neuroreport*, 7: 2925–2928.
- Neeper, S.A., Gómez-Pinilla, F., Choi, J. and Cotman, C.W. (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res.*, 726: 49–56.
- Nelson, R.L., Guo, Z., Halagappa, V.M., Pearson, M., Gray, A.J., Matsuoka, Y., Brown, M., Martin, B., Iyun, T., Maudsley, S., Clark, R.F. and Mattson, M.P. (2007) Prophylactic treatment with paroxetine ameliorates behavioral deficits and retards the development of amyloid and tau pathologies in 3xTgAD mice. *Exp. Neurol.*, 205: 166–176.
- Newcomer, J.W., Selke, G., Melson, A.K., Hershey, T., Craft, S., Richards, K. and Alderson, A.L. (1999) Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Arch. Gen. Psychiatry*, 56: 527–533.

- Newton, S.S., Thome, J., Wallace, T.L., Shirayama, Y., Schlesinger, L., Sakai, N., Chen, J., Neve, R., Nestler, E.J. and Duman, R.S. (2002) Inhibition of cAMP response element-binding protein or dynorphin in the nucleus accumbens produces an antidepressant-like effect. *J. Neurosci.*, 22: 10883–10890.
- Nibuya, M., Morinobu, S. and Duman, R.S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.*, 15: 7539–7547.
- Nissinen, M.J., Karlstedt, K., Castren, E. and Panula, P. (1995) Expression of histidine decarboxylase and cellular histamine-like immunoreactivity in rat embryogenesis. *J. Histochem. Cytochem.*, 43: 1241–1252.
- Nissinen, M.J. and Panula, P. (1995) Developmental patterns of histamine-like immunoreactivity in the mouse. *J. Histochem. Cytochem.*, 43: 211–227.
- Nobin, A., Baumgarten, H.G., Björklund, A., Lachenmayer, L. and Stenevi, U. (1973) Axonal degeneration and regeneration of bulbo-spinal indolamine neurons after 5,6-dihydroxytryptamine treatment. *Brain Res.*, 56: 1–24.
- Ochi, J. and Shimizu, K. (1978) Occurrence of dopamine-containing neurons in the midbrain raphe nuclei of the rat. *Neurosci. Lett.*, 8: 317–320.
- O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J. and Molliver, M.E. (1988) Methylenedioxymphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. *J. Neurosci.*, 8: 2788–2803.
- O'Hearn, E. and Molliver, M.E. (1984) Organization of raphe-cortical projections in rat: a quantitative retrograde study. *Brain Res. Bull.*, 13: 709–726.
- Olin, J.T., Katz, I.R., Meyers, B.S., Schneider, L.S. and Lebowitz, B.D. (2002) Provisional diagnostic criteria for depression of Alzheimer disease: rationale and background. *Am. J. Geriatr. Psychiatry*, 10: 129–141.
- Otake, K. (2005) Cholecystokinin and substance P immunoreactive projections to the paraventricular thalamic nucleus in the rat. *Neurosci. Res.*, 51: 383–394.
- Ownby, R.L., Crocco, E., Acevedo, A., John, V. and Loewenstein, D. (2006) Depression and risk for Alzheimer disease: systematic review, meta-analysis, and meta-regression analysis. *Arch. Gen. Psychiatry*, 63: 530–538.
- Palmer, A.M., Francis, P.T., Benton, J.S., Sims, N.R., Mann, D.M., Neary, D., Snowden, J.S. and Bowen, D.M. (1987) Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. *J. Neurochem.*, 48: 8–15.
- Parsons, A.A. (1991) 5-HT receptors in human and animal cerebrovasculature. *Trends Pharmacol. Sci.*, 12: 310–315.
- Pasqualotto, B.A., Hope, B.T. and Vincent, S.R. (1991) Citrulline in the rat brain: immunohistochemistry and coexistence with NADPH-diaphorase. *Neurosci. Lett.*, 128: 155–160.
- Pau, K.Y., Yu, J.H., Lee, C.J. and Spies, H.G. (1998) Topographic localization of neuropeptide Y mRNA in the monkey brainstem. *Regul. Pept.*, 75–76: 145–153.
- Paxinos, G. and Franklin, K.B.J. (2001) *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, San Diego, CA.
- Paxinos, G. and Watson, C. (1997) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego, CA.
- Paykel, E.S., Brugha, T. and Fryers, T. (2005) Size and burden of depressive disorders in Europe. *Eur. Neuropsychopharmacol.*, 15: 411–423.
- Petrov, T., Krukoff, T.L. and Jhamandas, J.H. (1992) The hypothalamic paraventricular and lateral parabrachial nuclei receive collaterals from raphe nucleus neurons: a combined double retrograde and immunocytochemical study. *J. Comp. Neurol.*, 318: 18–26.
- Petrov, T., Krukoff, T.L. and Jhamandas, J.H. (1994) Chemically defined collateral projections from the pons to the central nucleus of the amygdala and hypothalamic paraventricular nucleus in the rat. *Cell Tissue Res.*, 277: 289–295.
- Phillips, H.S., Hains, J.M., Armanini, M., Laramée, G.R., Johnson, S.A. and Winslow, J.W. (1991) BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron*, 7: 695–702.
- Pierce, E.T., Foote, W.E. and Hobson, J.A. (1976) The efferent connection of the nucleus raphe dorsalis. *Brain Res.*, 107: 137–144.
- Pomara, N., Greenberg, W.M., Branford, M.D. and Doraiswamy, P.M. (2003) Therapeutic implications of HPA axis abnormalities in Alzheimer's disease: review and update. *Psychopharmacol. Bull.*, 37: 120–134.
- Prolla, T.A. and Mattson, M.P. (2001) Molecular mechanisms of brain aging and neurodegenerative disorders: lessons from dietary restriction. *Trends Neurosci.*, 24: S21–S31.
- Ramboz, S., Oosting, R., Amara, D.A., Kung, H.F., Blier, P., Mendelsohn, M., Mann, J.J., Brunner, D. and Hen, R. (1998) Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc. Natl. Acad. Sci. U.S.A.*, 95: 14476–14481.
- Rapp, M.A., Schnaider-Beeri, M., Grossman, H.T., Sano, M., Perl, D.P., Purohit, D.P., Gorman, J.M. and Haroutunian, V. (2006) Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. *Arch. Gen. Psychiatry*, 63: 161–167.
- Ren-Patterson, R.F., Cochran, L.W., Holmes, A., Sherrill, S., Huang, S.J., Tolliver, T., Lesch, K.P., Lu, B. and Murphy, D.L. (2005) Loss of brain-derived neurotrophic factor gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities of serotonin transporter knockout mice. *J. Neurosci. Res.*, 79: 756–771.
- Richards, J.G. (1978) Cytochemistry and autoradiography in the search for transmitter-specific neuronal pathways. In: Coupland R.E. and Forssmann W.G. (Eds.), *Peripheral Neuroendocrine Interaction*. Springer, Heidelberg, pp. 1–14.
- Richards, J.G., Lorez, H.P. and Tranzer, J.P. (1973) Indolealkylamine nerve terminals in cerebral ventricles: identification by electron microscopy and fluorescence histochemistry. *Brain Res.*, 57: 277–288.
- Rodrigo, J., Springall, D.R., Uttenthal, O., Bentura, M.L., Abadia-Molina, F., Riveros-Moreno, V., Martínez-Murillo, R., Polak, J.M. and Moncada, S. (1994) Localization of nitric

- oxide synthase in the adult rat brain. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 345: 175–221.
- Rosel, P., Arranz, B., San, L., Vallejo, J., Crespo, J.M., Urretavizcaya, M. and Navarro, M.A. (2000) Altered 5-HT_{2A} binding sites and second messenger inositol trisphosphate (IP₃) levels in hippocampus but not in frontal cortex from depressed suicide victims. *Psychiatry Res.*, 99: 173–181.
- Rosel, P., Arranz, B., Urretavizcaya, M., Oros, M., San, L. and Navarro, M.A. (2004) Altered 5-HT_{2A} and 5-HT₄ post-synaptic receptors and their intracellular signalling systems IP₃ and cAMP in brains from depressed violent suicide victims. *Neuropsychobiology*, 49: 189–195.
- Rosel, P., Arranz, B., Vallejo, J., Oros, M., Crespo, J.M., Menchon, J.M. and Navarro, M.A. (1998) Variations in [3H]imipramine and 5-HT_{2A} but not [3H]paroxetine binding sites in suicide brains. *Psychiatry Res.*, 82: 161–170.
- Rupniak, N.M. and Kramer, M.S. (1999) Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK1) antagonists. *Trends Pharmacol. Sci.*, 20: 485–490.
- Russo-Neustadt, A., Beard, R.C. and Cotman, C.W. (1999) Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology*, 21: 679–682.
- Russo-Neustadt, A.A., Beard, R.C., Huang, Y.M. and Cotman, C.W. (2000) Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. *Neuroscience*, 101: 305–312.
- Sadikot, A.F. and Parent, A. (1990) The monoaminergic innervation of the amygdala in the squirrel monkey: an immunohistochemical study. *Neuroscience*, 36: 431–447.
- Saito, S., Kobayashi, S., Ohashi, Y., Igarashi, M., Komiya, Y. and Ando, S. (1994) Decreased synaptic density in aged brains and its prevention by rearing under enriched environment as revealed by synaptophysin contents. *J. Neurosci. Res.*, 39: 57–62.
- Sakai, K. and Crochet, S. (2001) Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. *Neuroscience*, 104: 1141–1155.
- Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. and Hen, R. (2003) Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*, 301: 805–809.
- Schumacher, J., Jamra, R.A., Becker, T., Ohlraun, S., Klopp, N., Binder, E.B., Schulze, T.G., Deschner, M., Schmal, C., Hofels, S., Zobel, A., Illig, T., Propping, P., Holsboer, F., Rietschel, M., Nothen, M.M. and Cichon, S. (2005) Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biol. Psychiatry*, 58: 307–314.
- Segal, M. (1977) Afferents to the entorhinal cortex of the rat studied by the method of retrograde transport of horseradish peroxidase. *Exp. Neurol.*, 57: 750–765.
- Segal, M. and Landis, S. (1974) Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. *Brain Res.*, 78: 1–15.
- Severin, E.S. and Kondratyev, A.D. (1988) Regulation of differentiation of PC12 cells by nerve growth factor. *Adv. Enzyme Regul.*, 27: 357–370.
- Sheline, Y.I., Gado, M.H. and Kraemer, H.C. (2003) Untreated depression and hippocampal volume loss. *Am. J. Psychiatry*, 160: 1516–1518.
- Sheline, Y.I., Wang, P.W., Gado, M.H., Csernansky, J.G. and Vannier, M.W. (1996) Hippocampal atrophy in recurrent major depression. *Proc. Natl. Acad. Sci. U.S.A.*, 93: 3908–3913.
- Shirayama, Y., Chen, A.C., Nakagawa, S., Russell, D.S. and Duman, R.S. (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.*, 22: 3251–3261.
- Sim, L.J. and Joseph, S.A. (1993) Dorsal raphe nucleus efferents: termination in peptidergic fields. *Peptides*, 14: 75–83.
- Sims, K.B., Hoffman, D.L., Said, S.I. and Zimmerman, E.A. (1980) Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: an immunocytochemical study. *Brain Res.*, 186: 165–183.
- Siuciak, J.A., Lewis, D.R., Wiegand, S.J. and Lindsay, R.M. (1997) Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol. Biochem. Behav.*, 56: 131–137.
- Skofitsch, G. and Jacobowitz, D.M. (1985) Immunohistochemical mapping of galanin-like neurons in the rat central nervous system. *Peptides*, 6: 509–546.
- Smith, M.A., Makino, S., Kvetnansky, R. and Post, R.M. (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.*, 15: 1768–1777.
- Soares, J.C. and Mann, J.J. (1997) The functional neuroanatomy of mood disorders. *J. Psychiatr. Res.*, 31: 393–432.
- Sohal, R.S. and Weindruch, R. (1996) Oxidative stress, caloric restriction, and aging. *Science*, 273: 59–63.
- Steinbusch, H.W. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6: 557–618.
- Steinbusch, H.W. (1984) Serotonin-immunoreactive neurons and their projections in the CNS. In: Bjorklund A. and Hokfelt T. (Eds.), *Handbook of Chemical Neuroanatomy*, Vol. 3. Elsevier, New York, pp. 68–125.
- Steinbusch, H.W., Nieuwenhuys, R., Verhofstad, A.A. and van der, K.D. (1981) The nucleus raphe dorsalis of the rat and its projection upon the caudatoputamen. A combined cytoarchitectonic, immunohistochemical and retrograde transport study. *J. Physiol. (Paris)*, 77: 157–174.
- Steinbusch, H.W.M. and Nieuwenhuys, R. (1982) Localization of serotonin-like immunoreactivity in the central nervous system and pituitary of the rat, with special references to the innervation of the hypothalamus. In: Haber B. and Gabay S. (Eds.), *Serotonin: Current Aspects of Neurochemistry and Function*. Plenum Press, New York, pp. 7–35.
- Stockmeier, C.A., Mahajan, G.J., Konick, L.C., Overholser, J.C., Jurjus, G.J., Meltzer, H.Y., Uyllings, H.B., Friedman, L. and Rajkowska, G. (2004) Cellular changes in the

- postmortem hippocampus in major depression. *Biol. Psychiatry*, 56: 640–650.
- Stratford, T.R. and Wirtshafter, D. (1990) Ascending dopaminergic projections from the dorsal raphe nucleus in the rat. *Brain Res.*, 511: 173–176.
- Svenningsson, P., Chergui, K., Rachleff, I., Flajolet, M., Zhang, X., El Yacoubi, M., Vaugeois, J.M., Nomikos, G.G. and Greengard, P. (2006) Alterations in 5-HT_{1B} receptor function by p11 in depression-like states. *Science*, 311: 77–80.
- Svenningsson, P. and Greengard, P. (2007) p11 (S100A10): an inducible adaptor protein that modulates neuronal functions. *Curr. Opin. Pharmacol.*, 7: 27–32.
- Swanwick, G.R., Kirby, M., Bruce, I., Buggy, F., Coen, R.F., Coakley, D. and Lawlor, B.A. (1998) Hypothalamic-pituitary-adrenal axis dysfunction in Alzheimer's disease: lack of association between longitudinal and cross-sectional findings. *Am. J. Psychiatry*, 155: 286–289.
- Szeszko, P.R., Lipsky, R., Mentschel, C., Robinson, D., Gunduz-Bruce, H., Sevy, S., Ashtari, M., Napolitano, B., Bilder, R.M., Kane, J.M., Goldman, D. and Malhotra, A.K. (2005) Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation. *Mol. Psychiatry*, 10: 631–636.
- Taber, E., Brodal, A. and Walberg, F. (1960) The raphe nuclei of the brain stem in the cat. I. Normal topography and cytoarchitecture and general discussion. *J. Comp. Neurol.*, 114: 161–187.
- Tejani-Butt, S.M., Yang, J. and Pawlyk, A.C. (1995) Altered serotonin transporter sites in Alzheimer's disease raphe and hippocampus. *Neuroreport*, 6: 1207–1210.
- Tork, I. (1990) Anatomy of the serotonergic system. *Ann. N.Y. Acad. Sci.*, 600: 9–34.
- Tsai, S.J. (2003) Brain-derived neurotrophic factor: a bridge between major depression and Alzheimer's disease? *Med. Hypotheses*, 61: 110–113.
- Uhl, G.R., Goodman, R.R., Kuhar, M.J., Childers, S.R. and Snyder, S.H. (1979) Immunohistochemical mapping of enkephalin containing cell bodies, fibers and nerve terminals in the brain stem of the rat. *Brain Res.*, 166: 75–94.
- Valentino, R.J., Bey, V., Pernar, L. and Commons, K.G. (2003) Substance P acts through local circuits within the rat dorsal raphe nucleus to alter serotonergic neuronal activity. *J. Neurosci.*, 23: 7155–7159.
- Valentino, R.J. and Commons, K.G. (2005) Peptides that fine-tune the serotonin system. *Neuropeptides*, 39: 1–8.
- Van Bockstaele, E.J., Biswas, A. and Pickel, V.M. (1993) Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res.*, 624: 188–198.
- Van Bockstaele, E.J. and Pickel, V.M. (1993) Ultrastructure of serotonin-immunoreactive terminals in the core and shell of the rat nucleus accumbens: cellular substrates for interactions with catecholamine afferents. *J. Comp. Neurol.*, 334: 603–617.
- van de Kar, L.D. and Lorens, S.A. (1979) Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. *Brain Res.*, 162: 45–54.
- van der Kooy, D. and Hattori, T. (1980a) Dorsal raphe cells with collateral projections to the caudate-putamen and substantia nigra: a fluorescent retrograde double labeling study in the rat. *Brain Res.*, 186: 1–7.
- van der Kooy, D. and Hattori, T. (1980b) Bilaterally situated dorsal raphe cell bodies have only unilateral forebrain projections in rat. *Brain Res.*, 192: 550–554.
- Vertes, R.P. (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J. Comp. Neurol.*, 313: 643–668.
- Wallace, T.L., Stellitano, K.E., Neve, R.L. and Duman, R.S. (2004) Effects of cyclic adenosine monophosphate response element binding protein overexpression in the basolateral amygdala on behavioral models of depression and anxiety. *Biol. Psychiatry*, 56: 151–160.
- Wang, Q.P., Guan, J.L. and Nakai, Y. (1995) Distribution and synaptic relations of NOS neurons in the dorsal raphe nucleus: a comparison to 5-HT neurons. *Brain Res. Bull.*, 37: 177–187.
- Wang, Q.P., Ochiai, H. and Nakai, Y. (1992) GABAergic innervation of serotonergic neurons in the dorsal raphe nucleus of the rat studied by electron microscopy double immunostaining. *Brain Res. Bull.*, 29: 943–948.
- Waselus, M., Galvez, J.P., Valentino, R.J. and Van Bockstaele, E.J. (2006) Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum. *J. Chem. Neuroanat.*, 31: 233–242.
- Wiklund, L., Leger, L. and Persson, M. (1981) Monoamine cell distribution in the cat brain stem. A fluorescence histochemical study with quantification of indolaminergic and locus coeruleus cell groups. *J. Comp. Neurol.*, 203: 613–647.
- Wolf, S.A., Kronenberg, G., Lehmann, K., Blankenship, A., Overall, R., Staufenbiel, M. and Kempermann, G. (2006) Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. *Biol. Psychiatry*, 60: 1314–1323.
- Wotherspoon, G., Albert, M., Ratray, M. and Priestley, J.V. (1994) Serotonin and NADPH-diaphorase in the dorsal raphe nucleus of the adult rat. *Neurosci. Lett.*, 173: 31–36.
- Wu, Z.L., Ciallella, J.R., Flood, D.G., O'Kane, T.M., Bozyczko-Coyne, D. and Savage, M.J. (2006) Comparative analysis of cortical gene expression in mouse models of Alzheimer's disease. *Neurobiol. Aging*, 27: 377–386.
- Wyss, J.M., Swanson, L.W. and Cowan, W.M. (1979) A study of subcortical afferents to the hippocampal formation in the rat. *Neuroscience*, 4: 463–476.
- Xu, H., Chen, Z., He, J., Haimanot, S., Li, X., Dyck, L. and Li, X.M. (2006) Synergetic effects of quetiapine and venlafaxine in preventing the chronic restraint stress-induced decrease in cell proliferation and BDNF expression in rat hippocampus. *Hippocampus*, 16: 551–559.
- Xu, H., Qing, H., Lu, W., Keegan, D., Richardson, J.S., Chlan-Fourney, J. and Li, X.M. (2002) Quetiapine attenuates the immobilization stress-induced decrease of brain-derived

- neurotrophic factor expression in rat hippocampus. *Neurosci. Lett.*, 321: 65–68.
- Yager, J.Y., Wright, S., Armstrong, E.A., Jahraus, C.M. and Saucier, D.M. (2006) The influence of aging on recovery following ischemic brain damage. *Behav. Brain Res.*, 173: 171–180.
- Yamamoto, T. and Hirano, A. (1985) Nucleus raphe dorsalis in Alzheimer's disease: neurofibrillary tangles and loss of large neurons. *Ann. Neurol.*, 17: 573–577.
- Yoshida, K., McCormack, S., España, R.A., Crocker, A. and Scammell, T.E. (2006) Afferents to the orexin neurons of the rat brain. *J. Comp. Neurol.*, 494: 845–861.
- Zhang, X., Beaulieu, J.M., Sotnikova, T.D., Gainetdinov, R.R. and Caron, M.G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science*, 305: p. 217.
- Zhang, X., Gainetdinov, R.R., Beaulieu, J.M., Sotnikova, T.D., Burch, L.H., Williams, R.B., Schwartz, D.A., Krishnan, K.R. and Caron, M.G. (2005a) Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron*, 45: 11–16.
- Zhang, X., Gainetdinov, R.R., Beaulieu, J.M., Sotnikova, T.D., Burch, L.H., Williams, R.B., Schwartz, D.A., Krishnan, K.R. and Caron, M.G. (2005b) Response to correspondence: loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron*, 48: 705–706.
- Zobel, A., Joe, A., Freymann, N., Clusmann, H., Schramm, J., Reinhardt, M., Biersack, H.J., Maier, W. and Broich, K. (2005) Changes in regional cerebral blood flow by therapeutic vagus nerve stimulation in depression: an exploratory approach. *Psychiatry Res.*, 139: 165–179.
- Zweig, R.M., Ross, C.A., Hedreen, J.C., Steele, C., Cardillo, J.E., Whitehouse, P.J., Folstein, M.F. and Price, D.L. (1988) The neuropathology of aminergic nuclei in Alzheimer's disease. *Ann. Neurol.*, 24: 233–242.

CHAPTER 13

Dynamics of the dopaminergic system as a key component to the understanding of depression

Gal Yadid* and Alexander Friedman

Neuropharmacology Laboratory, The Mina and Everard Goodman Faculty of Life Sciences and the Leslie and Susan Gonda (Goldshmid) Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat-Gan 52900, Israel

Abstract: For decades, clinical treatment of depression has usually involved antidepressants that target noradrenergic and serotonergic neurotransmission. Over the past half century, no genuinely groundbreaking progress has been made in the pharmacological development of antidepressant drugs. Dopaminergic mesolimbic and mesocortical systems are involved in hedonia and motivation, two core symptoms of depression. However, their role in the pathophysiology of depression and their manipulation to treat depression has received little attention. Recent findings indicate the potential usefulness of monitoring limbic dopaminergic dynamics in combination with mathematical analysis. In this chapter comprehensive review of data from animal models, genetics, neuroimaging and human clinical trials that strengthen the case for dopaminergic dysfunction in the pathophysiology of major depression. This chapter focuses on recent convergence of data describing the fluctuation in activity of the mesolimbic dopaminergic system, and discusses its crucial role in manifestation of depressive-like behavior. Decoding the functionality of the dopaminergic system is important to the understanding of depression and the development of future efficient antidepressant treatments.

Keywords: depressive behaviour; nucleus accumbens; ventral tegmental area; dopamine; cellular activity

Introduction

For decades, clinical treatment of depression has usually involved antidepressants that target noradrenergic and serotonergic neurotransmission. Over the past half century, no genuinely groundbreaking progress has been made in the pharmacological development of antidepressant drugs. Dopaminergic mesolimbic and mesocortical systems are involved in hedonia and motivation, two core symptoms of depression.

However, their role in the pathophysiology of depression and their manipulation to treat depression has received little attention. Convergence of data from animal models and human clinical trials strengthen the case for dopaminergic dysfunction in the pathophysiology of major depression.

Recent findings indicate the potential usefulness of monitoring limbic dopaminergic dynamics in combination with mathematical analysis. In this chapter we discuss the role of dopaminergic system dynamics in depression. Decoding the functionality of the dopaminergic system may help to understand depression and development of future antidepressant drugs.

*Corresponding author. Tel.: +972 3 531 8123;
Fax: +972 3 635 4965; E-mail: yadidg@mail.biu.ac.il

Implication of the mesolimbic dopamine system in depressive behaviour

Several new lines of evidence implicate the mesolimbic dopamine (DA) system originating in the ventral tegmental area (VTA), in the pathogenesis and treatment of depression (Dunlop and Nemeroff, 2007; Gershon et al., 2007). Dopaminergic neurons label environmental stimuli with appetitive value, as well as, predict rewards and motivating events (Schultz and Dickinson, 2000). Since anhedonia and loss of motivation are core characteristics of depression (Kapur and Mann, 1992), altered limbic DA neurotransmission may be involved in depression. In fact, the following discussion suggests involvement of the mesolimbic DA system in depression and response to chronic antidepressant therapies.

Studies in humans

Pharmacological interventions that block or decrease DA, are associated with the induction and deepening of depression (Kapur and Mann, 1992; Ordway and Mann, 2002). After acute administration of sulpiride (D2-like receptor antagonist) (Cervo and Samanin, 1988; Willner et al., 2005), depressed patients successfully treated with serotonin selective reuptake inhibitors (SSRIs), display increased mood and psychomotor symptoms associated with depression (Willner et al., 2005). Conversely, DA agonists can mimic antidepressant effects (Willner et al., 2005). Several dopaminergic agonists usually used to treat Parkinson's disease, were tried also on depressed patients in some open trials as well as randomized controlled trials alone or as adjuvant treatment and compared with classic antidepressants.

Bromocriptine, piribedil and pramipexole, D2-like agonists and pergolide and amantadine, less specific DA agonists, have antidepressant effects (Vale et al., 1971; Shopsin and Gershon, 1978; Waehrens and Gerlach, 1981; Bouras and Bridges, 1982; Theohar et al., 1982; Izumi et al., 2000). Other dopaminergic drugs that affect DA bioavailability at the synapse also have been reported to improve depression. A selective DA reuptake inhibitor, nomifensine (Kapur and

Mann, 1992) and methylphenidate (El Mallakh, 2000) which stimulate DA release were reported to be an effective antidepressant. It is interesting to note that the antidepressants bupropion and venlafaxine have DA reuptake inhibitor activity (Kapur and Mann, 1992; Stahl, 2000).

In accordance, reduced DA transported (DAT) density and elevated DA receptor D2/D3 receptor binding in the central and basal nuclei of the amygdala of postmortem depressed subjects compared with psychiatrically normal controls was reported (Pare, 1969). However, another study failed to show changes in D2 density (Papp et al., 1994). These reports were consistent with neuroimaging that found elevated striatal D2-binding levels in depressed patients (Post et al., 1978; Parsey et al., 2001; Perugi et al., 2001).

No difference in the allelic distributions of D4 receptor, DAT and COMT polymorphisms was found (Gordon et al., 1996). While one meta-analysis by Lopez et al. (2005) found that the D4 2-repeat allele increases the risk of depressive symptomatology, others have not found any association between D4 alleles and mood disorders (Serretti et al., 2002).

Studies in animal models

Abnormalities in dopaminergic receptor within the limbic areas of the brain were also observed in several different animal models of depression.

Kram et al. (2002) found an increase in D1-like and decrease in D2-like receptor density in learned helplessness rats, which suggest a decrease in extracellular DA in this model of depression (Meyer et al., 2001). However, no clinical studies reported a difference in D1 receptor binding between depressed subjects who died by suicide and controls (Pare, 1969; Papp et al., 1994).

Few reports have shown that chronic antidepressant treatment increases D2-like binding activity in the limbic system of rats. Maj et al. showed that chronic treatment with imipramine, amitriptyline, mianserin or fluoxetine increased binding to the D2-like receptor and D3-like receptor in limbic areas (Klimek and Maj, 1989; Maj et al., 1996, 1998; Rogoz and Dziedzicka-Wasylewska, 1999; Lammers et al., 2000). D2-like receptor antagonists

blocked the action of antidepressants on anhedonia expressed by mild chronic stress, an animal model of depression (Muscat et al., 1992). This might parallel the anhedonia observed in depressed patients (Muscat et al., 1990). Another rat model of depression demonstrated that immobility in the forced swim test can be reduced by desipramine, imipramine or amitriptyline. These effects are blocked by injection of sulpiride to the nucleus accumbens (NAc) (Cervo and Samanin, 1988) but not to the caudate-putamen (Cervo and Samanin, 1987). Conversely, chronic D2-like

agonists, quinpirole and bromocriptine, can mimic both the behavioural activity effects of antidepressants in the sucrose consumption model (Maj et al., 2000).

Monoamines levels in the limbic system of Flinders sensitive line (FSL) rats, an animal model of depression were markedly elevated. Daily treatment for 14 days with desipramine normalized these abnormal levels and the abnormal depressive-like behaviour (Zangen et al., 2001) (Fig. 1A). This result may be interpreted as a hypofunctionality of the DA system since the two drugs mentioned above

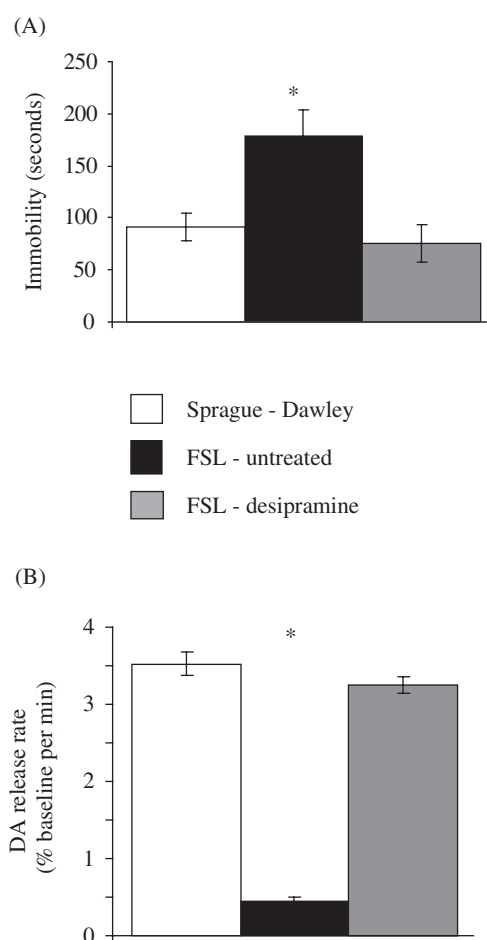


Fig. 1. Effect of desipramine on depressive behaviour and dopamine release in the accumbens. Desipramine at a dose and treatment course that effects electrophysiology also effects immobility of FSL rats in a swim test ($F[2, 27] = 3.2$, $p < 0.05$, one way ANOVA; panel A) and the rate of GBR-12909-mediated DA accumulation in dialysates from the accumbens. Bar indicates switch of CSF perfused via microdialysis probe to CSF containing GBR-12909 (10 μM). Dialysates were collected in 30-min intervals ($F[2, 27] = 4.05$, $p < 0.05$, one way ANOVA; panel B). Adapted with permission from Friedman et al. (2007).

that increase DA bioavailability at the synapse, nomifensine and tranylcypromine also increased D2-like receptor binding (Martin et al., 1995).

Taken together, these findings suggest that chronic administration of antidepressant drugs of different classes share a common effect of increasing DA cell activity and binding activity of DA D2-like receptors in the NAc. This increased activity has functional correlates both in the response to dopaminergic and D2-like agonist drugs as well as in behavioural models of depression (Gershon et al., 2007).

Basal extracellular levels of DA and metabolites

In the abovementioned imaging studies, changes in receptor binding may reflect increased numbers of receptors in depression, an increase in affinity of the receptor for the ligand, or a decrease in DA release into the synapse.

More direct evidence proposed impaired DA release in contribution to the pathophysiology of depression. In some studied levels of homovanillic acid (HVA), the major metabolite of DA, are reduced in the cerebrospinal fluid (CSF) of depressed patients (Kapur and Mann, 1992; Reddy et al., 1992). Moreover, depressed patients exhibited reduced striatal dopaminergic activity compared with healthy volunteers, using a positron emission tomography (PET) study and [^{18}F]-fluorodopa (Pruessner et al., 2004). Transcranial magnetic stimulation applied to the frontal cortex increases extracellular DA concentrations in the striatum of depressed individuals (D'haenen and Bossuyt, 1994; DeBattista et al., 2000).

Extracellular levels of DA and its main metabolites were determined using the microdialysis technique in FSL rats, an animal model of depression. Although the tissue content (which represents both extracellular and intracellular levels) of DA in the NAc of FSL rats has been shown to be six to seven times higher than in control rats (Zangen et al., 1997, 1999a; Yadid et al., 2000a), the basal extracellular levels of DA and its main metabolites, DOPAC and HVA in the NAc of FSL rats were found to be 40–50% lower than those in control rats (Fig. 2). This may indicate

a relatively decreased dopaminergic firing or increased uptake of DA in the depressed rats. The rate of DA release was determined, by the application of a blocker of DA transporter. By perfusing GBR 12909, an inhibitor of DA reuptake, through the microdialysis probe into the accumbens shell, one may be able to monitor the rate of DA release by its accumulation in the extracellular space. A significant decrease was detected in the rate of DA release in the extracellular space of FSL rats compared to Sprague–Dawley (SD) rats (Fig. 1B). Administration of desipramine for 14 days caused increases in rates of DA release in FSL rats to levels similar to those observed in the control SD rats, whereas the metabolism of the serotonergic system seem to be normal.

However, the basal extracellular levels of serotonin (5-HT) and its metabolite 5-HIAA in the NAc of FSL rats were not significantly different from the control rats (Fig. 2). These results suggest that FSL rats have decreased extracellular DA release and turnover in the NAc compared to SD rats.

Association between depressive behaviour and absence of serotonin–dopamine interaction in the nucleus accumbens

The basic symptoms characterizing the depressed patient are anhedonia and lack of motivation, both of which are expressed in the absence of an immediate response to environmental stimuli (Jefferson and Griest, 1994). Since DA release in the NAc is associated with motivation and hedonia (Koob and Bloom, 1988; Self and Nestler, 1995; Wise, 1996; Schultz et al., 1997), the lower basal extracellular levels of DA observed in the NAc of FSL rats (Fig. 2) could explain their impaired motivation and anhedonia. However, chronic treatment with desipramine or paroxetine, which normalize the behavioural deficiency (e.g. the immobility score) of FSL rats, do not necessarily normalize the extracellular levels of DA in the NAc (Yadid et al., 2000a). Desipramine-induced elevation of basal DA levels was observed in microdialysis studies (Tanda et al., 1994). Number of studies show that fluoxetine (Bymaster et al., 2002; Koch et al., 2002) and

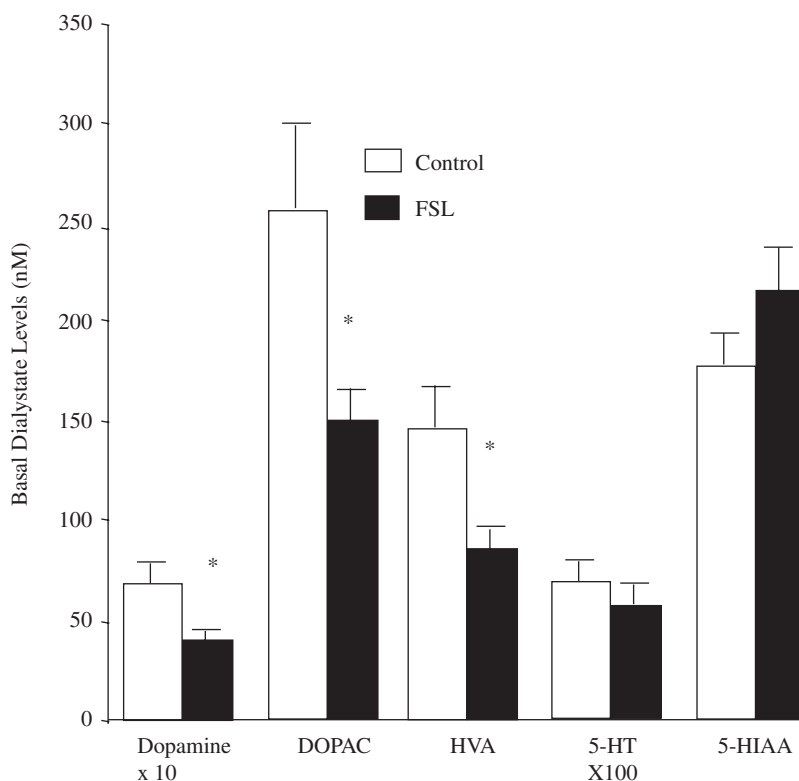


Fig. 2. Basal monoamine levels in dialysates obtained from the nucleus accumbens of Flinders sensitive line (FSL) and control rats. Microdialysis was performed in the nucleus accumbens of FSL and control rats. Basal monoamine levels in dialysates obtained from freely moving rats were measured by means of high performance liquid chromatography. Mean \pm SEM values of 10 rats in each group are presented. Significantly different values were detected using the *t*-test * $p < 0.01$. Adapted with permission from Zangen et al. (2001).

paroxetine (Nakayama, 2002) elevate extracellular DA. However, other studies found that chronic paroxetine decreases (Zangen et al., 1999b) and fluoxetine (Tanda et al., 1994) does not affect extracellular DA levels in the NAc. Other studies suggest that some members of the new generation of antidepressants, mirtazapine and venlafaxine, increase DA release in the NAc of FSL rats (Dremencov et al., 2006), same as found by Nakayama et al. (2004) and Weikop et al. (2004).

Therefore, no correlation is found between basal extracellular DA levels in the NAc and depressive behaviour of FSL rats. The absence of correlation between depressive behaviour of FSL rats and the basal extracellular monoamine levels in the NAc led us to seek stimulated rather than basal levels.

Local application of 5-HT into the NAc of SD rats can stimulate local DA release (Parsons and

Justice, 1993; De Deurwaerdere et al., 1998). A blunt effect of 5-HT on extracellular DA levels was observed in FSL rats (Fig. 3). Chronic treatment with the antidepressants desipramine or paroxetine, which almost abrogates the behavioural deficits of FSL rat, normalized the interaction between 5-HT and DA in the NAc (Fig. 4A). The observation that the 5-HT-DA interaction in the control rats was not significantly affected by chronic antidepressant treatment (Fig. 4B) is compatible with the observations demonstrating that the behaviour of normal rats and the mood of healthy human volunteers are not affected by antidepressant treatment (Overstreet, 1993; Jefferson and Griest, 1994; Yadid et al., 2000a). Since the release of DA in the NAc was shown to be essential for reward and motivation, it was proposed that the inability of 5-HT to induce

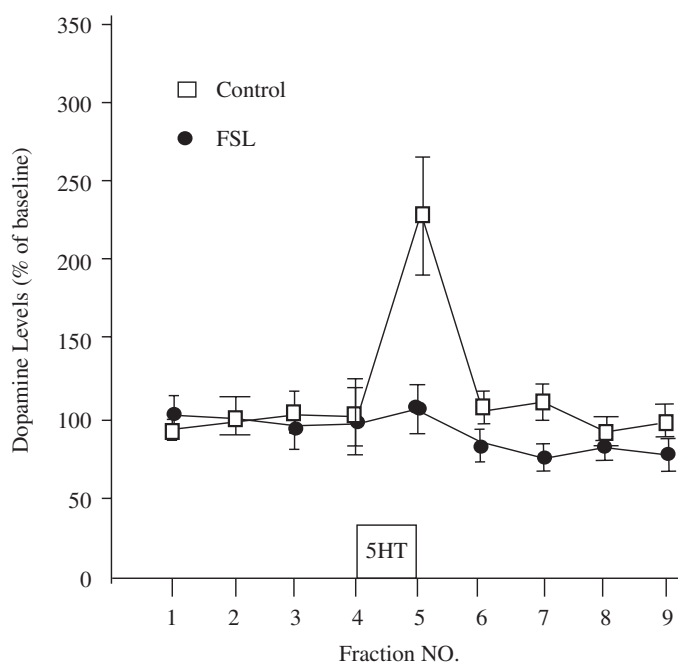


Fig. 3. Effect of local application of serotonin (5-HT) on extracellular levels of dopamine in the nucleus accumbens of Flinders sensitive line (FSL) and control rats. The microdialysis probe was perfused with artificial cerebrospinal fluid (aCSF) before and after a 30-min perfusion with aCSF containing $0.5 \mu\text{M}$ 5-HT. Dialysates were collected at 30-min intervals. The mean dopamine level measured in the four dialysates prior to the 5-HT perfusion was used as the baseline dopamine level. Mean \pm SEM values of 10 rats in each group are presented. Values statistically different from baseline were determined by means of analysis of variance with repeated measures over time followed by the Student–Newman–Keuls post-hoc test. * $p < 0.01$. Adapted with permission from Zangen et al. (2001).

DA release in FSL rats may account for their behavioural deficiencies. Chronic (but not acute) treatment with antidepressant drugs, which is necessary to affect depressive behaviour (American Psychiatric Association, 1993; Overstreet, 1993; Jefferson and Griest, 1994), normalizes the 5-HT–DA interaction, probably by affecting synaptic plasticity (Nestler and Duman, 1995).

Alteration in serotonin receptors contribute to lower DA bioavailability and manifestation of depressive behaviour

Further observations indicated that impaired 5-HT–DA interaction in the NAc is an essential factor in the aetiology of depression, and its normalization by chronic antidepressant treatment gives a new perspective on the mode of action of these drugs.

Clinical studies showed that antidepressants require varying minimal treatment periods before the appearance of a therapeutic effect, which is called onset time. The pharmacological mechanism that enables some antidepressants to act faster than others is poorly understood. It is possible to conclude that the onset time of an antidepressant drug depends on the activation of specific monoamine pathways and may involve several neurotransmitters and their receptors. Several studies suggest that fast-onset antidepressants have mixed serotonergic (5-HT) and noradrenergic effects (Blier, 2001). Antidepressant onset time may be shortened by coadministration with pindolol, a β -adrenergic and 5-HT_{1A} receptor antagonist (Artigas et al., 2001; Blier, 2001), suggesting an involvement of 5-HT_{1A} receptors in the fast-onset phenomena (Blier and Bergeron, 1995; Blier and Bergeron, 1998; Briner and Dodel, 1998). Other studies support the hypothesis that fast-onset

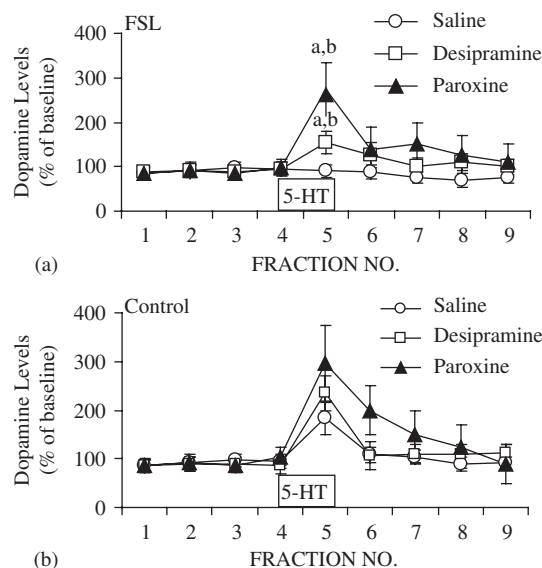


Fig. 4. Effect of antidepressant treatment on the serotonin (5-HT)–dopamine interaction in the nucleus accumbens of Flinders sensitive line (FSL) and control rats. FSL or control rats were chronically treated with desipramine (5 mg/kg/day), paroxetine (7.5 mg/kg/day) or saline for 18 days. Microdialysis was performed as described in Fig. 4. The effect of artificial cerebrospinal fluid (aCSF) containing 0.5 μ M 5-HT was tested in all groups. Mean \pm SEM values of eight rats in each group are presented. Values statistically different from baseline (a) or differences between the saline and drug-treated groups (b) were determined by means of analysis of variance followed by the Student–Newman–Keuls post-hoc test. $p < 0.01$. No significant differences were observed between desipramine- and paroxetine-treated rats. Adapted with permission from Zangen et al. (2001).

drugs have 5-HT₂ receptor-specific actions (Bakish et al., 1997). 5-HT stimulates local DA release (Parsons and Justice, 1993; Zangen et al., 2001) in the NAc via the activation of the 5-HT_{1A} (Campbell and McBride, 1995) and 5-HT₃ (Ichikawa and Meltzer, 1999) receptors. The excitatory-like effect of those receptors on DA release is balanced by the inhibitory effect of 5-HT_{2C} receptors (Di Matteo et al., 2001). The major difference between desipramine and nefazodone is in their affinities for 5-HT₂ receptors; the affinity of desipramine for 5-HT_{2A} receptor is only 1.8% that of nefazodone. Additionally, nefazodone is a potent 5-HT_{2C} antagonist (Sanchez and Hyttel, 1999). Therefore, the antagonist action of nefazodone on 5-HT_{2A/2C} receptors may explain the fast onset of nefazodone compared with desipramine in some studies (Dremencov et al., 2004a). Nefazodone also has a lower potency for norepinephrine reuptake than desipramine. However, the antidepressant paroxetine has even lower norepinephrine reuptake inhibition affinity than nefazodone but

has not demonstrated fast-onset action in the FSL model of depression (Yadid et al., 2000b) or, to the best of our knowledge, in clinical studies. Furthermore, onset of restoration of 5-HT-induced DA release in the NAc correlates with the onset of behavioural improvements after antidepressant treatment. Accordingly, antidepressants which demonstrate faster onset of behavioural improvement (such as venlafaxine and mirtazapine) are associated with normalization of impaired 5-HT-induced DA release (Dremencov et al., 2004a).

Further studies elucidated the roles of the critical role of 5-HT_{2C} receptors in the fast-onset phenomenon. It was proposed that the increased inhibitory activity of accumbal 5-HT_{2C} receptors might be involved in disabling 5-HT-induced DA release in FSL rats, and that this correlates with depressive-like behaviour (Dremencov et al., 2006). Other 5-HT receptors such as 5-HT₃ receptors may also contribute to the changes in 5-HT-induced DA release (Dremencov et al., 2006). Further, the finding that antidepressants which are effective

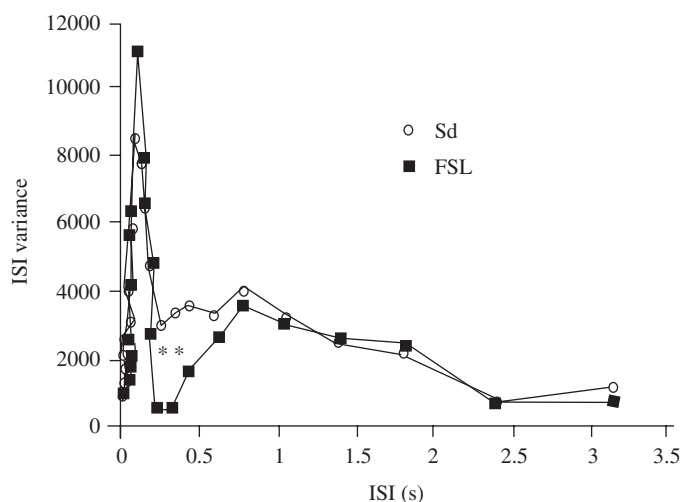


Fig. 5. Variance of interspike intervals (ISIs) for the control Sprague–Dawley and Flinders sensitive line (FSL) rats. The variance between the number of events in each ISI partitioning for FSL ($n = 12$) and the control Sprague–Dawley ($n = 14$) rats was calculated. The difference between variances of the number of events in each ISI partitioning was tested using an F -test ($F[1, 24] = 5.85$, $p < 0.005$). Adapted with permission from Friedman et al. (2005).

in restoring the activity of the 5-HT_{2C} receptor to the levels of controls, raises the possibility that antidepressant drugs with a high affinity for the 5-HT_{2C} receptor are promising candidates for restoring 5-HT-induced DA release and improving depressive-like behaviour in a shorter time course than other drugs (Dremencov et al., 2006). Thus, antidepressant drugs that will block 5-HT_{2C} and activate 5-HT₃ receptors will probably restore 5-HT-induced DA release in the NAc and normalize depressive-like behaviour faster than classical antidepressant drugs (Dremencov et al., 2004a, 2005). This suggestion agrees with clinical studies that demonstrated that mirtazapine, which acts on 5-HT_{2C} and 5-HT₃ receptors, and nefazodone, which acts on 5-HT_{2C}, and whose metabolite acts on 5-HT₃ receptors, are characterized by a more rapid onset of behavioural effects of treatment (Artigas et al., 2002).

Variability of the mesolimbic neuronal activity in a rat model of depression

The negative correlation between tissue content and extracellular DA in the accumbens may suggest a

decreased cell-firing in the VTA. The absolute refractory period of VTA dopaminergic neurons is 2.5 ms (Friedman et al., 2005, 2007, 2008). The longest, single, interspike interval (ISI) recorded from the VTA was about 3.5 s. The interval between 2.5 ms and 3.5 s was split into 23 logarithmically spaced partitionings and histograms were constructed, for the number of events in each partitioning for each recording. The variance between the number of events in each ISI partitioning for three groups, untreated FSL rats, FSL rats treated with desipramine and control SD rats, was studied. Figure 5 shows the variance between the number of events as a function of the length of the ISI for the control and FSL rats. ISIs recorded from both the control and FSL rats showed homogeneous distribution between the affinity histograms. The mean firing rate did not differ between the control SD and FSL rats. For both groups, the variance of ISI was maximal at the ISI length of 0.1 s, and a gradual reduction in the longer ISI of about 3 s. However, FSL rats showed a significant decrease in the variance of ISIs for lengths of 0.26–0.35 s.

Furthermore, it was found that 14-day treatment with desipramine, which normalized

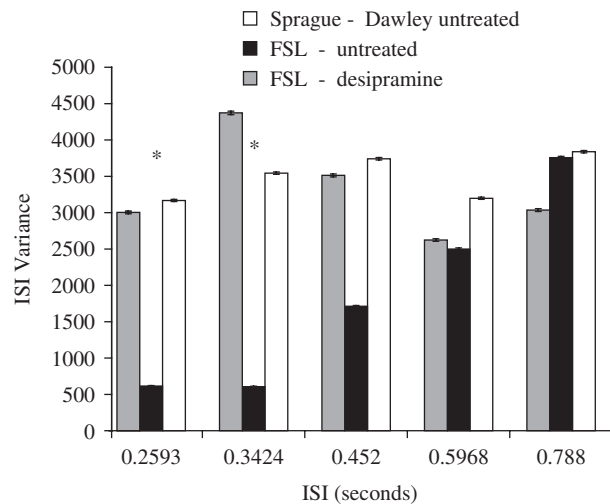


Fig. 6. Chronic desipramine treatment normalizes the variance of interspike intervals (ISIs) in Flinders sensitive line (FSL) rats. The variance of 0.3-s-long ISIs is presented for untreated Sprague–Dawley ($n = 14$), FSL rats ($n = 12$) and FSL rats treated with desipramine ($n = 8$) for 14 days. Untreated FSL rats showed a decrease in the variance of 0.3-s-long ISIs ($F[1, 18] = 0.14$, $p < 0.005$). However, FSL rats treated with desipramine showed a variance of 0.3-s-long ISIs equal to the results of the control Sprague–Dawley rats. Adapted with permission from Friedman et al. (2005).

depressive like behaviour in FSL rats, also increased the variance of ISIs of 0.26–0.35 s to the ranges of the control SD animals (Fig. 6).

This finding can be explained by the fact that DA-cell firing in the VTA is altered in an FSL animal model of depression and corrected by desipramine. The details of this finding will be demonstrated in the following section.

VTA firing and their alteration in depressive-like behaviour

Two modes of DA cells firing

Individual neurons within the mesolimbic system developed specialised intrinsic membrane properties that have led them to be typically defined as either single spiking or high frequency burst-firing neurons. The bursting mode may be used to boost the gain of neural signalling of important or novel events by enhancing transmitter release and dendritic depolarization. Conversely, the single-spiking mode may be used to reduce neuronal signalling and could be associated with habituation to unimportant events (Cooper, 2002).

However, these classifications are not fixed because under certain circumstances, when triggered by appropriate input, action potential output can switch back and forth between the two modes. It is not clear at present what information is coded by the switch in output modes (Cooper, 2002).

Dopaminergic neurons fire tonically or, conversely, irregularly at frequencies around 3–7 Hz (Grace and Bunney, 1984). VTA dopaminergic neurons fire in both burst and single-spike modes. Each burst event can be characterized by its length (number of spikes) or duration. Bursting activity of Dopaminergic cells induces greater DA release at the terminal site than that caused by the single-spiking mode (Cooper, 2002). In addition, bursting activity corresponds to synaptic plasticity in the VTA during reward-related learning (Schultz and Dickinson, 2000).

It was shown that the VTA of SD rats has the capability of firing bursts with a large amount of spikes, while FSL rats rarely show this pattern. A significant alteration in burst-like and cluster activities was detected in FSLs, which was restored by desipramine treatment. Initial comparison of SD and desipramine-treated FSL rats appeared to illustrate similar firing modes, whereas more

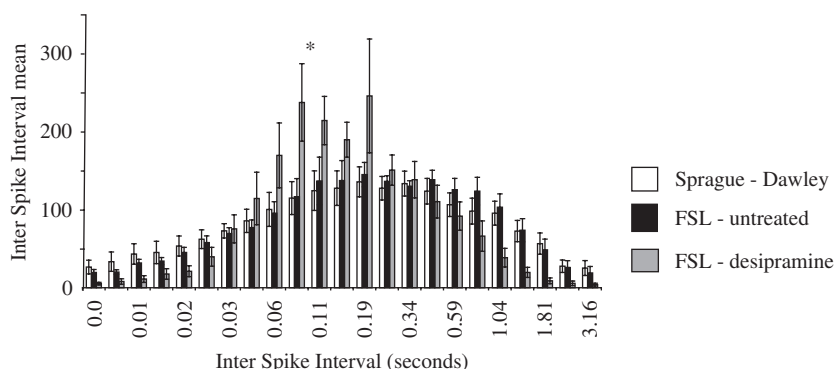


Fig. 7. Interspike intervals distribution histogram. A significant difference was found between desipramine-treated FSL rats and FSL and control groups ($F[2, 27]$, $*p < 0.05$; one way ANOVA). Adapted with permission from Friedman et al. (2007).

rigorous analysis revealed that the firing patterns differed. Desipramine treatment, which normalized depressive-like behaviour, corrected but did not fully normalize the dopaminergic mesolimbic activity.

Antidepressants correct but do not normalize neuronal firing activity

Electrophysiological activity from the VTA was recorded and interspike intervals from the recording were analysed. The absolute refractory period of VTA dopaminergic neurons is 2.5 ms (Grace and Bunney, 1984). The longest, single, ISI recorded from the VTA was approximately 3.5 s. Mean cell frequencies were approximately 4–5.5 Hz corresponding to ISI 0.18–0.25 s. Mean frequencies did not differ significantly between the FSL, FSL treated with desipramine and control SD rats.

An ISI histogram was constructed for each group (Fig. 7). The ISI histograms for SD and untreated FSL groups are essentially similar, but the histogram for the desipramine-treated FSL group shows an increased activity in the 0.04–0.19 s interval. Additionally, the histogram mean of the desipramine-treated FSL group is lower than that of the non-treated groups, demonstrating that desipramine treatment increases neuronal activity.

As can be seen in the histogram (Fig. 7), desipramine treatment also reduced the variability of the ISI, which suggests that the randomness

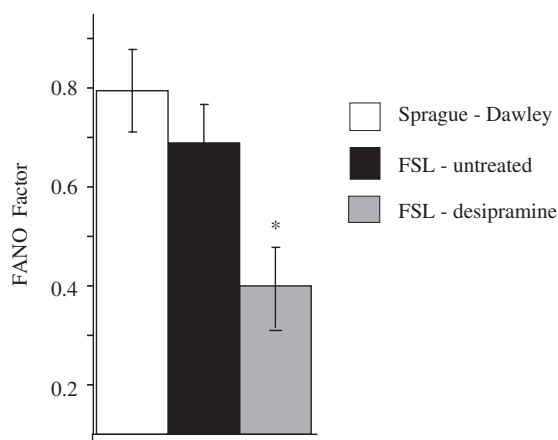


Fig. 8. FANO factor of neuron firing. The burst series demonstrated a poissonic process measured by FANO factor ($*p < 0.02$ [$F[2, 27] = 4.9$, $p < 0.02$ one-way ANOVA, FSL rats vs. the desipramine-treated FSL and control groups). Adapted with permission from Friedman et al. (2007).

of the process decreases during treatment. This observation was tested by calculating the FANO factor of the ISI data for each group. It was found that this parameter is relatively stable with the average variation of approximately 0.1. Thus, this parameter can be used to measure dopaminergic neuronal activity. The mean FANO factor was calculated for each group (Fig. 8), and it was found that the FANO factor of all three groups is less than one, indicating that the firing is not totally random. This would imply that dopaminergic firing is not dependant only on the firing rate. It was

observed that the FANO factor of desipramine-treated FSL rats was significantly lower than that of the non-treated FSL and control SD rats. Furthermore, treatment of FSL rats distanced this factor from the value found for the SD control group, more than before treatment. On the other hand, depressive-like behaviour of the desipramine-treated FSL rats, as measured by immobility in the swim test, improved following treatment. This indicates that the randomness of firing does not correlate with correction of the behavioural manifestation of depression. Therefore, other features of neuronal activity that would correlate with the behaviour of the animals were searched for.

In order to find such features, the neuronal activity was split into burst and single firing modes and respective histograms were constructed for each of the three groups. A burst was defined as a sequence of spikes starting with an ISI < 80 ms and ending with the concurrence of two spikes with an ISI > 160 ms (Grace and Bunney, 1984). In addition, we separated the short bursts (two to four spikes in each burst) from the long bursts. It was found that the burst activity significantly correlates with the depressive-like behaviour of the animals, while the single-firing mode did not show correlation with this behaviour (data not shown). Untreated FSL rats have a greater number of short bursts than control rats, and conversely, control rats have a greater number of long bursts (Fig. 9).

Desipramine treatment of FSL rats caused an increase in the amount of long burst firing, similar to its amount in SD rats. On the other hand, in Figs. 7 and 8 we observed that the neuronal activity of desipramine-treated FSL rats significantly differed from that of the healthy SD control group. Further analysis of Fig. 7 shows increased activity in the post burst area corresponding to ISI by 0.16–0.26 s. This can be considered as a cluster, defined as a sequence of spikes starting with an ISI < 80 ms and ending with the concurrence of two spikes with an ISI > 260 ms. The clusters consist of a burst and ‘burst tail’, which is defined as the post-burst activity (Fig. 11). Treatment of FSL rats with desipramine significantly increased the generation of ‘burst tails’ and thus generated more clusters. The percentage of short bursts and single spikes that attached as a cluster was calculated

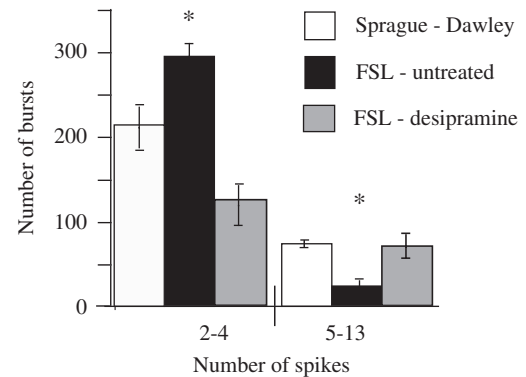


Fig. 9. Burst distribution histogram. Burst distribution histogram analysis of VTA electrophysiology showed differences between Sprague-Dawley, FSL rats and desipramine-treated FSL rats ($n = 10$ cells; number of cells and rats are equal; two way ANOVA (strains $F[2, 7] = 33.7$, $p < 0.001$; burst type $F[2, 7] = 53.4$, $p < 0.001$; interactions $F[2, 7] = 55.4$, $p < 0.001$). * $p < 0.001$ versus corresponding group. Adapted with permission from Friedman et al. (2007).

for each group of rats (Fig. 10A, B). Desipramine-treated FSL rats demonstrated 25% more bursts and single spikes that were attached to clusters than control and untreated-FSL rats. Since the variability of the ISI data in a cluster is small, the existence of a large amount of clusters decreases the FANO factor (Fig. 8).

Firing patterns of dopamine and depression-like behaviour

The underlying hypothesis is that the bursting-like activity of VTA dopaminergic neurons is essential for a beneficial response to commonly used antidepressants. It was found that the VTA of SD rats has the capability of firing bursts with a large amount of spikes, while FSL rats rarely show this pattern (Fig. 9). In SD rats, the average firing was calculated as 5 spikes/burst with an 80–160 ms interval between spikes. However, FSL rats compensate for this inability by firing an increased number of small bursts calculated as 3 spikes/burst with an 80–160 ms interval between spikes. It was hypothesized that a lower amount of long bursts in VTA neuronal activity may induce depressive states, since release of DA which is important for reward and a feeling of well-being requires many long bursts.

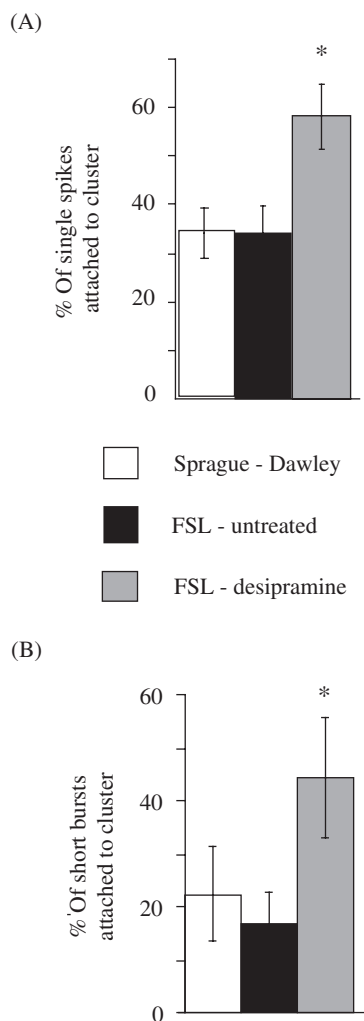


Fig. 10. Cluster characteristics. Percentage of single spikes (A) or bursts (B) attached to the calculated cluster. * p value < 0.01 FSL rats versus desipramine-treated FSL rats and control groups ($F[2, 27] = 4.8$, one way ANOVA) for single spikes analysis. * p value < 0.04 ($F[2, 27] = 3.7$, one way ANOVA) for burst analysis. Adapted with permission from Friedman et al. (2007).

Desipramine treatment of FSL rats caused an increase in the amount of long burst firing, similar to its amount in SD rats. Thus, desipramine treatment probably corrected the inability of the FSL rat VTA to fire bursts with a large amount of spikes. On the other hand, the neuronal activity of desipramine-treated FSL rats significantly differed from that of controls (Figs. 7 and 8). This difference was elucidated by the increased activity seen in the post-burst area corresponding to ISI within 0.16–0.26 s. It was suggested that desipramine treatment of FSL rats induced the dopaminergic cells to compensate for reduced long-burst activity by attaching several short bursts and single spikes to the end of a burst. This created a cluster consisting of a short burst and 'burst tail', defined as the post-burst activity. A model of VTA firing and occurrence of clusters is suggested in Fig. 11.

The integration of short bursts and creation of clusters in desipramine-treated FSLs may have caused the observed elevations in NAc DA to control levels. Concurrently with increased DA levels, desipramine-treated FSL rats also show a degree of immobility similar to controls, thus implying that the burst compensation can alleviate the behavioural manifestations of depression.

Stimulus-bound dopaminergic neuronal output has been suggested to track the earliest predictors of hedonia by phasically bursting. Conversely, the single-spiking mode may be used to dampen neuronal signalling and may be associated with habituation to unimportant events (Cooper, 2002). However, these classifications are not fixed because under certain circumstances, when triggered by appropriate input, action potential output can switch back and forth between the two modes. It is not clear at present what information is coded during the switch in output modes

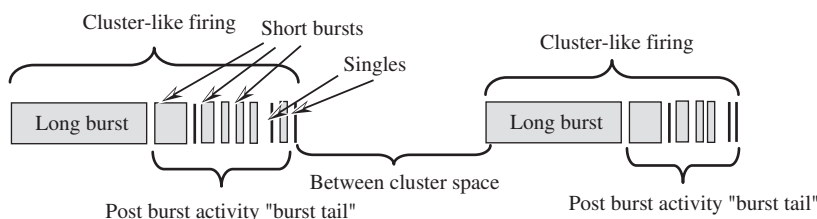


Fig. 11. Cluster model illustration. Adapted with permission from Friedman et al. (2007).

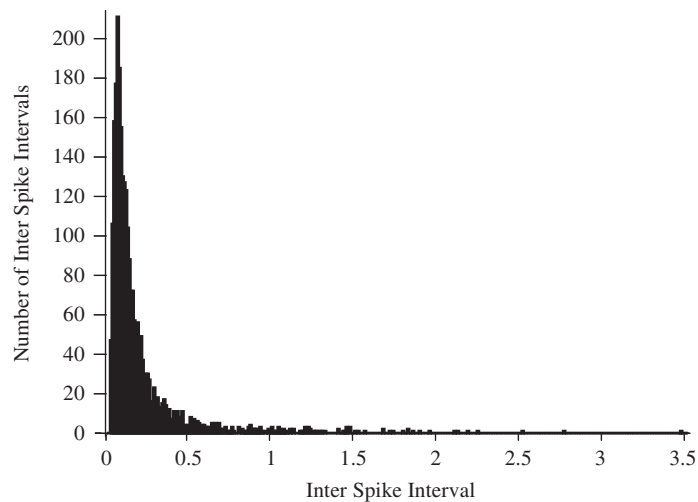


Fig. 12. Interspike interval distribution histogram for representative 'bursting' cell. The early peak appearance indicates bursting. Adapted with permission from Friedman et al. (2007).

(Cooper, 2002). This discussion attempted to shed light on single burst switching and its importance in behavioural manifestation of depression.

Knowing that bursting enhances and prolongs signal strength is important for understanding the communication between individual neurons in general and in relation to complex memorized behavioural-related tasks. Dopaminergic burst firing has an irregular tonic/phasic nature. A number of brain regions have a periodic firing nature, such as regions that coordinate heart or respiratory processing. The current study demonstrates the periodic nature of dopaminergic bursting in the VTA which functions as a motivational and reward intersection. Interestingly, periodicity was found in the burst sequence and was not a time-dependent process.

Cell firing in the VTA has burst sequence periodicity

The ISI distribution histogram for each cell recording (see one cell representative example in Fig. 12) was calculated. Several DA-like cells exhibit firing in burst mode. The high peak (0.08–0.1 s ISIs) in the histogram shows a greater number of small ISI intervals that belong to bursts. Periodicity was

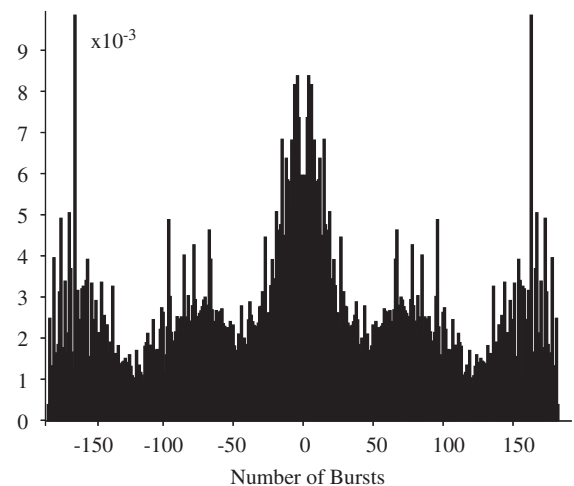


Fig. 13. The autocorrelation of a representative cell. Periodicity after 80 bursts is demonstrated. Adapted with permission from Friedman et al. (2007).

observed only in the burst mode. Therefore, the burst firing was separated from single spikes firing.

Bursts were characterized by using a new parameter termed 'burst influence'. Burst influence was calculated for each individual burst and a burst influence sequence was generated for each cell. The autocorrelation of a representative cell is shown in Fig. 13, which indicates periodicity after 80 bursts. In addition, the Fast Fourier Transform

(FFT) and power spectrum for a sequence of the 'burst influence' was calculated (Fig. 14A–C) and periodicity of the DA burst firing in 20% of bursting neurons was found. This periodicity was found in the burst sequence and is not dependent on time. It is important to note that this burst firing process has frequencies of 0.1–0.15 Hz, which are lower than heart and respiratory rates, and hence could not be explained by external biological sources.

These data suggest a mechanism of 'neuronal cell memory' of specific burst sequences. It was hypothesized that loops in neuronal networks may be explained by such periodic burst firing. Since this process is demonstrated in the VTA, it may further suggest that loops have an important role in reward, reinforcement, motivational and decision-making processes. Understanding depression on the cellular level has great potential for developing new antidepressant treatments and construction of novel therapeutic methods.

Decoding of dopaminergic mesolimbic activity and depressive behaviour

Mathematical description of ISIs, single spikes and bursts

Cell bursting-like activity is correlated with the functional dynamics of the accumbal DA release. Therefore, an analysis was performed, on the burst dynamics recorded from the VTA of FSL and control rat, separately from the whole firing time series (ISIs). Since the ability of a burst to increase DA release from nerve terminals depends both on burst length and on the neuron-firing rate within a given burst (Cooper, 2002), a mathematical descriptive parameter was introduced. This mathematical parameter was defined as burst length (number of spikes) multiplied by the firing rate within the burst (number of spikes/s). This mathematical parameter may be also defined as the burst influence. This burst influence was calculated for each individual burst. Burst influence series were analysed by phase-space dimensions algorithm. The stability of phase-space dimensions for each animal at non-intersecting

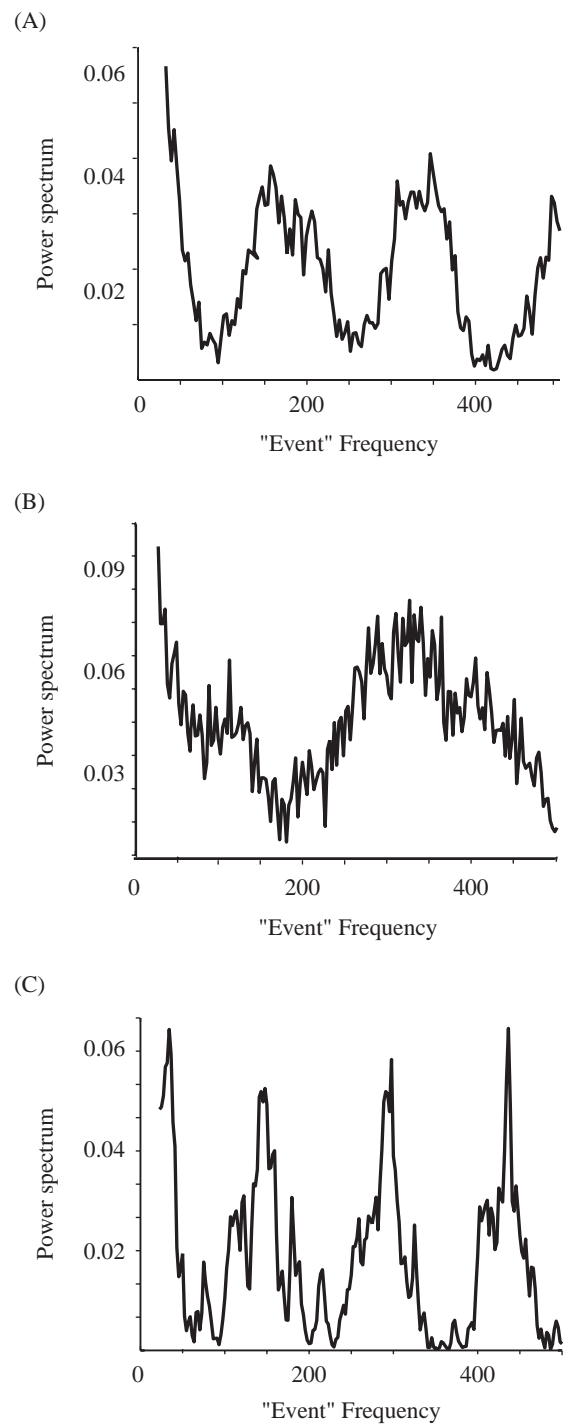


Fig. 14. Periodicity of bursting Panels A–C: Representative demonstration of power spectrum of burst influence. Adapted with permission from Friedman et al. (2007).

Table 1. Characteristics of ISI, DA release and behavioural parameters

Characteristics	Sprague–Dawley (controls)	FSL	FSL + desipramine ^a
A. Burst characteristics			
Mean burst length (no. of spikes)	4.90 ± 0.69 ^b	4.11 ± 1.51 ^b	4.73 ± 0.79 ^b
Mean burst duration (s)	0.50 ± 0.16 ^b	0.54 ± 0.16 ^b	0.59 ± 0.24 ^b
B. Variances			
GBR 12909-induced DA release	155093.71 ^c + 82016.77 ^d – 20548.34 ^d	1435.44 ^{c,e} + 569.319 ^d – 142.64 ^d	232978.1 ^c + 154004.56 ^d – 38584.03 ^d
ISI (0.2–0.4 s)	3400.64 ^b + 5767.23 ^d – 1444.91 ^d	600.96 ^{b,e} + 887.04 ^d – 222.24 ^d	3687.98 ^b + 3412.99 ^d – 855.09 ^d
C. Phase–space dimensions ^f			
Full ISI time-series	10.33 ± 1.57 ^b	13.18 ± 1.19 ^b	12.38 ± 1.35 ^b
Single-spike events	10.29 ± 1.12 ^b	12.91 ± 0.5 ^b	13.17 ± 1.22 ^b
Burst	6.21 ± 2.15 ^b	0.82 ± 0.05 ^{b,e}	5.6 ± 2.01 ^b
D. Dialysate DA levels (nM)	6.7 ± 1.24 ^g	3.9 ± 1 ^{g,e}	9.8 ± 1.04 ^g
E. DA release rate (% baseline/min) ^f	3.52 ± 0.15 ^c	0.45 ± 0.05 ^{c,e}	3.25 ± 0.1 ^c
F. Immobility (s) ^f	91 ± 13 ^{g,h}	178 ± 26 ^{g,h}	83 ± 20 ^{g,h}
G. GBR 12909-induced DA levels (nM)	34.3 ± 10.3 ^c	7.6 ± 1.4 ^{c,e}	39.1 ± 7.1 ^c

^a5 mg/kg i.p. for 14 days.^b10 rats, 10 cells.^c10 rats.^dConfidence value.^eSignificant difference between FSL and two other groups $p < 0.05$, one way ANOVA for mean and F -test for variance.^fA linear correlation dependence between the phase–space dimension of bursting-like activity, immobility and speed of DA release (% baseline/min) was observed (Pearson test $r = 0.9973$).^gNumber of rats in group — 10.^hSwim test performed 22–24 h after last injection of desipramine.

time intervals was verified prior to further analysis. Since it was found relatively stable (average variation ≤ 0.7), it was used to measure the neuronal activity. The mean phase–space value was calculated for the three types of time-series (whole ISI, single-spike events and sequence of the bursts) for the three experimental groups.

The phase–space dimensions of entire VTA ISI time series and of single spike events were larger (indicating highly chaotic processes) than that of bursting-like activity (indicating non-linear determinism) (Table 1C). For FSL, desipramine-treated FSL and SD rats, the phase–space dimensions of the ISI time series and single-spike events, were similar. However, the phase–space dimensions of the bursting-like activity of FSL and SD rats were significantly different. Treatment of the FSL rats with desipramine for 14 days increased their bursting-like activity to levels similar to those of the control SD rats.

Correlation between neuronal firing, DA release rate and depressive behaviour

It was demonstrated that a selective DA reuptake inhibitor, GBR 12009 (Sarre et al., 2004), was

administered into the accumbens alter DA accumulation and was affected by altered DA reuptake or impaired tonic regulation (Grace, 1991). The correlation between VTA-cell firing and accumbal DA release in an animal model for depression was examined. The rate of GBR 12909-mediated DA accumulation in the NAc and phase–space dimension of bursting-like activity of dopaminergic neurons in the VTA was significantly lower in FSL rats than in the SD controls. Chronic treatment of FSL rats with desipramine for 14 days raised the values of these parameters in FSL rats to levels comparable to those of the control SD rats. A linear correlation dependence between the phase–space dimension of bursting-like activity and rate of GBR 12909-induced DA accumulation was observed.

A three-dimensional correlation between neuronal firing, DA release and depressive behaviour in FSL, desipramine-treated FSL and SD rats was determined using phase–space dimensions of bursting-like activity, microdialysis and immobility during swim test data (Fig. 15). A high correlation of these three variables was observed between the three experimental groups.

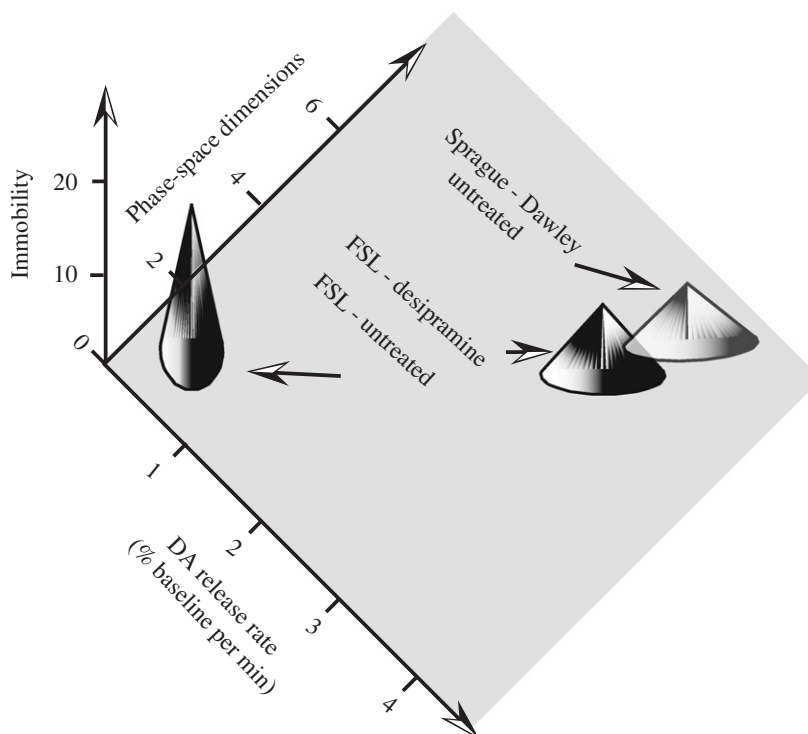


Fig. 15. Correlation between neuronal firing, rate of DA release and depressive behaviour. Neuronal firing was determined by phase-dimension of bursting-like activity in the VTA, rate of the GBR 12909-mediate DA release by microdialysis was determined in the NAc, and depressive behaviour by immobility in a swim test. Adapted with permission from Friedman et al. (2007).

Mechanism of VTA activity and depressive behaviour

The underlying hypothesis is that the bursting-like activity of VTA dopaminergic neurons is essential for a beneficial response to currently used antidepressants. It was found that the bursting-like activity in the VTA is highly deterministic, signal regulated by a relatively small number of parameters, unlike the chaotic-like single-spiking activity, which has random features (Table 1C). The chaotic character of the neuronal activity in the VTA can be explained by highly complex network of auto- and cross-regulation of dopaminergic neurons (Adell and Artigas, 2004), as well as by stochastic processes in the neuron membrane, such as channel noise (Steinmetz et al., 2000). FSL rats displayed a decreased dimension complexity of bursting-like activity of VTA dopaminergic cells when

compared to the SD rats used as controls. This parameter was restored by repetitive antidepressant. Chronic administration of the antidepressant desipramine to FSL rats elevated the dimensional complexity of this activity to levels observed in SD rats. A correlation was also observed between firing of neurons in the VTA, the dynamics of DA release in the accumbens and depressive behaviour.

FSL rats, an animal model of depression, are characterized by a deficit in basal levels of DA release in the NAc (Zangen et al., 2001; Dremencov et al., 2004b). This may indicate a possible lower phasic activity of VTA cells. Moreover, lowering DA levels in terminal sites may increase DA synthesis and abnormal phasic responses to compensate for the decreased responses of the DA system (Floresco et al., 2003). Indeed a higher DA tissue content was observed in the NAc of FSL rats (Zangen et al., 2001). The release of

mesolimbic DA is regulated by a number of factors. Synaptic (phasic) levels are mediated primarily by bursting events of cell bodies and lead to much larger release than if these neuron fire irregular single spike mode (Schultz, 1998; Cooper, 2002; Floresco et al., 2003). Phasic-burst firing induces massive synaptic DA release (Cooper, 2002; Floresco et al., 2003), accompanied by rapidly DA removal by reuptake before escaping the synaptic cleft, whereas increased input of other neurons activity to the DA synapse modulates tonic extrasynaptic DA levels, which are less affected by reuptake processes (Floresco et al., 2003).

In fact, blocking DA reuptake at NAc synapses by GBR 12909 increased DA levels eightfold over baseline. Thus, phasic activity massively contributes to DA release in the NAc, but is masked by the fast and efficient reuptake of DA. Although presynaptic modulation cannot be excluded, a valid assumption may be made that DA accumulation due to blocking of DA reuptake characterizes dominantly the phasic release of DA in the NAc. The rate of accumulation of this accumbal DA can be compared with the DA cell firing in the VTA. Similarly, when DA reuptake is blocked by amphetamines, release of DA is phasic, not tonic (Floresco et al., 2003). Comparing the rate of VTA activity and the rate of DA release in the NAc, reveal that the efficacy of the mesolimbic DA pathway is decreased in FSL rats. It is worth to note that bursting-like activity in the VTA of rats is still observed under chloral-hydrate anaesthesia (Di Mascio et al., 1999; Dremencov et al., 2004a). This can be explained by high sensitivity of dopaminergic cells to spontaneous inputs arriving from the cortex (Cooper, 2002). However, this spontaneous bursting by intrinsic clock oscillations may occur in the membrane of dopaminergic cells and be facilitated by acetylcholine input from the pedunculopontine nucleus (Kitai et al., 1999).

Previously, it was shown that FSL rats are characterized by decreased variance of 0.2–0.4 s ISIs, a variance that is normalized by repetitive administration of desipramine (see section “Firing patterns of dopamine and depression-like Behavior”). Therefore, it is possible to postulate that variance of ISIs is relevant to the dynamics of DA release in limbic areas of the brain. Indeed a

correlation between the variance of 0.2–0.4 s ISIs and variance of DA release was found (Table 1B). In addition, FSL rats demonstrated monotonic firing of the DA VTA cells, with ISIs of 0.2–0.4 s. Thus, the parameter of ISI variance seems to be relevant to the dynamics of DA neurotransmission and prediction of antidepressant effects.

Based on phase–space dimension values, the conclusion was reached that FSL (depressed) rats are characterized by different type of bursting-like dynamics than normal (non-depressed) rats. The phase–space dimensions of bursting-like activity of FSL rats indicate a fully predictable linear process, while that of SD rats can be characterized as a more non-linear deterministic process (Baker et al., 1996). These findings indicate the potential usefulness of monitoring limbic dopaminergic dynamics during development of future antidepressant drugs.

Concluding remarks

Increasing data from depressive individuals and animal models indicate a role for the dopaminergic system in the manifestation of depressive behaviour. Although not yet in clinical use, interfering with the mesocorticolimbic activity seems to be an alternative promising attitude to treat depression. This could be achieved by developing specific medications that are directed toward specific serotonergic receptors to modulate DA outflow, or by directly activating selected DA receptors. Alternatively, dopaminergic cells may be electrically stimulated through an electrode implanted into the VTA. It is especially intriguing to apply a deep-brain-stimulation to the depressed brain in light of the presented data. If indeed a specific window of the cell activity is changed, and may be characterized as a dynamic pattern, then ‘copy and pasted’ of a programmed pattern, recorded from a normal brain, to the diseased brain may result in a switch from a depressed-state to a normal-state. As history of four decades of pharmacological intervention with this chronic disease did not prove fast-onset and long-term efficiency, electrical stimulation has immediate results (such as in Parkinson’s disease). However,

this possibility for depression and its log lasing still needs to be proved.

Abbreviations

DA	dopamine
DAT	dopamine transporter
FSL	Flinders sensitive line
5-HT	serotonin
5-HIAA	5-hydroxyindoleacetic acid
HVA	homovanillic acid
ISI	interspike interval
NAC	nucleus accumbens
VTA	ventral tegmental area

Appendix. Set of mathematical tools for neuronal firing dynamic analysis

PDF: probability density function

A probability density function of a random variable X is a non-negative function $f(x)$ that determines the probability density of the random variable. The probability that the random variable in question is in any particular interval $[a, b]$ is the integral of the function $f(x)$ from a to b . The histogram of a sample is used to estimate the *PDF*. In the present study, the random variable is the interspike interval (ISI) of the mesolimbic neuronal activity and different modes in it. Our observations are based on the comparison between the probability density functions, and more precisely the histograms, of the ISI data based on the neuronal activity and different modes of this activity collected for the three groups of rats. Since the value of the data is positive and close to zero, the logarithmic scale is used.

FANO factor

Spike firing process is a partially random process. The measure of randomness of such process can be determined by a Fano factor, which is defined as $F = (\sigma^2 W) / (\mu W)$, where $\sigma^2 W$ is the variance and μW the mean of a random process in a time window W . The FANO factor is equal to 1 if

the process is completely random with a given mean, called a Poisson process. If the process is completely regular, the Fano factor is 0.

In order to verify the stability of the FANO factor, it was calculated in non-intersecting time intervals for each rat. The factor was found to be relatively stable (average variation ≤ 0.1). Therefore, it was used to measure the randomness of the neuronal activity.

Periodicity detection

Information regarding the existence of periodicity of a time series within a system is important for understanding the system. The periodicity detection task is performed by time-domain methods or frequency-domain methods. Time-domain methods use autocorrelation functions while frequency-domain methods use spectral density functions. Each method has its limitations. We utilized information from both time-frequency and autocorrelation functions in our analysis.

Autocorrelation

Autocorrelation is a mathematical tool used for analysis of signals in time domain. Autocorrelation measures how well a signal matches a time-shifted version of itself, as a function of the size of the time shift. More precisely, it is the cross-correlation of a signal with itself. Autocorrelation is useful for finding repeating patterns in a signal even in the presence of noise.

Fast Fourier transform

The fast Fourier transform (FFT) is a discrete Fourier transform algorithm. The Fourier transform decomposes a function into a collection (spectrum) of its periodic components. Each periodic component is characterized by its frequency — the number of periods in a second measured in hertz. The spectral density, also termed as power spectral density or energy spectral density, is a distribution of the periodic component frequencies composing the signal. It is often simply designated as the spectrum of the signal. A peak in the power spectrum indicates a dominant

periodicity with the frequency corresponding to the position of the peak's maximum point.

Phase-space dimension

Phase-space dimensions were suggested by Grassberger and Procaccia (1983) as a tool to distinguish stochastic processes from deterministic chaotic time series (which may be low-dimensional or high-dimensional). Phase-space (or state space) dimensions describe the level of complexity of the system and allow representation of the behaviour of a system in a geometric form. The number of dimensions required for the phase space is a function of the 'degrees of freedom' of the system. A dynamical system consists of two parts: the notions of a state (the essential information about a system) and a dynamic (a rule that describes how the state evolves with time). This evolution can be visualized in a phase space.

References

- Adell, A. and Artigas, F. (2004) The somatodendritic release of dopamine in the ventral tegmental area and its regulation by afferent transmitter systems. *Neurosci. Biobehav. Rev.*, 28: 415–431.
- American Psychiatric Association. (1993) Practice guideline for major depressive disorder in adults. *Am. J. Psychiatry*, 150: 1–26.
- Artigas, F., Celada, P., Laruelle, M. and Adell, A. (2001) How does pindolol improve antidepressant action? *Trends Pharmacol. Sci.*, 22: 224–228.
- Artigas, F., Nutt, D.J. and Shelton, R. (2002) Mechanism of action of antidepressants. *Psychopharmacol. Bull.*, 36(Suppl. 2): 123–132.
- Baker, G.L., Gollub, J.P. and Blackburn, J.A. (1996) Inverting chaos: extracting system parameters from experimental data. *Chaos*, 6: 528–533.
- Bakish, D., Hooper, C.L., Thornton, M.D., Wiens, A., Miller, C.A. and Thibaut, C.A. (1997) Fast onset: an open study of the treatment of major depressive disorder with nefazodone and pindolol combination therapy. *Int. Clin. Psychopharmacol.*, 12: 91–97.
- Blier, P. (2001) Pharmacology of rapid-onset antidepressant treatment strategies. *J. Clin. Psychiatry*, 62(Suppl. 15): 12–17.
- Blier, P. and Bergeron, R. (1995) Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. *J. Clin. Psychopharmacol.*, 15: 217–222.
- Blier, P. and Bergeron, R. (1998) The use of pindolol to potentiate antidepressant medication. *J. Clin. Psychiatry*, 59(Suppl. 5): 16–23.
- Bouras, N. and Bridges, P.K. (1982) Bromocriptine in depression. *Curr. Med. Res. Opin.*, 8: 150–153.
- Briner, K. and Dodel, R.C. (1998) New approaches to rapid onset antidepressants. *Curr. Pharm. Des.*, 4: 291–302.
- Bymaster, F.P., Zhang, W., Carter, P.A., Shaw, J., Chernet, E., Phebus, L., Wong, D.T. and Perry, K.W. (2002) Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl.)*, 160: 353–361.
- Campbell, A.D. and McBride, W.J. (1995) Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens. *Pharmacol. Biochem. Behav.*, 51: 835–842.
- Cervo, L. and Samanin, R. (1987) Evidence that dopamine mechanisms in the nucleus accumbens are selectively involved in the effect of desipramine in the forced swimming test. *Neuropharmacology*, 26: 1469–1472.
- Cervo, L. and Samanin, R. (1988) Repeated treatment with imipramine and amitriptyline reduced the immobility of rats in the swimming test by enhancing dopamine mechanisms in the nucleus accumbens. *J. Pharm. Pharmacol.*, 40: 155–156.
- Cooper, D.C. (2002) The significance of action potential bursting in the brain reward circuit. *Neurochem. Int.*, 41: 333–340.
- DeBattista, C., Solvason, H.B., Breen, J.A. and Schatzberg, A.F. (2000) Pramipexole augmentation of a selective serotonin reuptake inhibitor in the treatment of depression. *J. Clin. Psychopharmacol.*, 20: 274–275.
- De Deurwaerdere, P., Stinus, L. and Spampinato, U. (1998) Opposite change of in vivo dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT₃ receptors. *J. Neurosci.*, 18: 6528–6538.
- D'haenen, H.A. and Bossuyt, A. (1994) Dopamine D₂ receptors in depression measured with single photon emission computed tomography. *Biol. Psychiatry*, 35: 128–132.
- Di Matteo, A., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22: 229–232.
- Di Mascio, M., Di Giovanni, G., Di Matteo, V. and Esposito, E. (1999) Decreased chaos of midbrain dopaminergic neurons after serotonin denervation. *Neuroscience*, 92: 237–243.
- Dremencov, E., Gispan-Herman, I., Rosenstein, M., Mendelman, A., Overstreet, D.H., Zohar, J. and Yadid, G. (2004a) The serotonin–dopamine interaction is critical for fast-onset action of antidepressant treatment: in vivo studies in an animal model of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28: 141–147.
- Dremencov, E., Nahshoni, E., Levy, D., Mintz, M., Overstreet, D.H., Weizman, A. and Yadid, G. (2004b) Dimensional complexity of the neuronal activity in a rat model of depression. *Neuroreport*, 15: 1983–1986.
- Dremencov, E., Newman, M.E., Kinor, N., Blatman-Jan, G., Schindler, C.J., Overstreet, D.H. and Yadid, G. (2005) Hyperfunctionality of serotonin-2C receptor-mediated inhibition of

- accumbal dopamine release in an animal model of depression is reversed by antidepressant treatment. *Neuropharmacology*, 48: 34–42.
- Dremencov, E., Weizmann, Y., Kinor, N., Gispan-Herman, I. and Yadid, G. (2006) Modulation of dopamine transmission by 5HT_{2C} and 5HT₃ receptors: a role in the antidepressant response. *Curr. Drug Targets.*, 7: 165–175.
- Dunlop, B.W. and Nemeroff, C.B. (2007) The role of dopamine in the pathophysiology of depression. *Arch. Gen. Psychiatry*, 64: 327–337.
- El Mallakh, R.S. (2000) An open study of methylphenidate in bipolar depression. *Bipolar. Disord.*, 2: 56–59.
- Floresco, S.B., West, A.R., Ash, B., Moore, H. and Grace, A.A. (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.*, 6: 968–973.
- Friedman, A., Deri, I., Friedman, Y., Dremencov, E., Goutkin, S., Kravchinsky, E., Mintz, M., Levi, D., Overstreet, D.H. and Yadid, G. (2007) Decoding of dopaminergic mesolimbic activity and depressive behavior. *J. Mol. Neurosci.*, 32: 72–79.
- Friedman, A., Dremencov, E., Crown, H., Levy, D., Mintz, M., Overstreet, D.H. and Yadid, G. (2005) Variability of the mesolimbic neuronal activity in a rat model of depression. *Neuroreport*, 16: 513–516.
- Friedman, A., Friedman, Y., Dremencov, E. and Yadid, G. (2008) VTA dopamine neuron bursting is altered in an animal model of depression and corrected by desipramine. *J. Mol. Neurosci.*, 34: 201–209.
- Gershon, A.A., Vishne, T. and Grunhaus, L. (2007) Dopamine D₂-like receptors and the antidepressant response. *Biol. Psychiatry*, 61: 145–153.
- Gordon, I., Weizman, R. and Rehavi, M. (1996) Modulatory effect of agents active in the presynaptic dopaminergic system on the striatal dopamine transporter. *Eur. J. Pharmacol.*, 298: 27–30.
- Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, 41: 1–24.
- Grace, A.A. and Bunney, B.S. (1984) The control of firing pattern in nigral dopamine neurons: single spike firing. *J. Neurosci.*, 4: 2866–2876.
- Grassberger, P. and Procaccia, I. (1983) Measuring the strangeness of strange attractors. *Phys. D*, 9: 189–208.
- Ichikawa, J. and Meltzer, H.Y. (1999) R(+)-8-OH-DPAT, a serotonin(1A) receptor agonist, potentiated S(–)-sulpiride-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens but not striatum. *J. Pharmacol. Exp. Ther.*, 291: 1227–1232.
- Izumi, T., Inoue, T., Kitagawa, N., Nishi, N., Shimanaka, S., Takahashi, Y., Kusumi, I., Odagaki, Y., Denda, K., Ohmori, T. and Koyama, T. (2000) Open pergolide treatment of tricyclic and heterocyclic antidepressant-resistant depression. *J. Affect. Disord.*, 61: 127–132.
- Jefferson, J.W. and Griest, J.H. (1994) Mood disorders. In: Hales R.E., Yudofsky S.C. and Talbott J.A. (Eds.), *Textbook of Psychiatry, Mood Disorders*. American Psychiatry Press, Washington, DC, pp. 465–494.
- Kapur, S. and Mann, J.J. (1992) Role of the dopaminergic system in depression. *Biol. Psychiatry*, 32: 1–17.
- Kitai, S.T., Shepard, P.D., Callaway, J.C. and Scroggs, R. (1999) Afferent modulation of dopamine neuron firing patterns. *Curr. Opin. Neurobiol.*, 9: 690–697.
- Klimek, V. and Maj, J. (1989) Repeated administration of antidepressants enhances agonist affinity for mesolimbic D₂-receptors. *J. Pharm. Pharmacol.*, 41: 555–558.
- Koch, S., Perry, K.W., Nelson, D.L., Conway, R.G., Threlkeld, P.G. and Bymaster, F.P. (2002) R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: an in vivo microdialysis and receptor binding study. *Neuropsychopharmacology*, 27: 949–959.
- Koob, G.F. and Bloom, F.E. (1988) Cellular and molecular mechanisms of drug dependence. *Science*, 242: 715–723.
- Kram, M.L., Karmer, G.L., Ronan, P.J., Steciuk, M. and Petty, F. (2002) Dopamine receptors and learned helplessness in the rat: an autoradiographic study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 26(4): 639–645.
- Lammers, C.H., Diaz, J., Schwartz, J.C. and Sokoloff, P. (2000) Selective increase of dopamine D₃ receptor gene expression as a common effect of chronic antidepressant treatments. *Mol. Psychiatry*, 5: 378–388.
- Lopez, L.S., Croes, E.A., Sayed-Tabatabaei, F.A., Claes, S., Van Broeckhoven, C. and van Duijn, C.M. (2005) The dopamine D₄ receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. *Biol. Psychiatry*, 57: 999–1003.
- Maj, J., Dziedzicka-Wasylewska, M., Rogoz, R. and Rogoz, Z. (1998) Effect of antidepressant drugs administered repeatedly on the dopamine D₃ receptors in the rat brain. *Eur. J. Pharmacol.*, 351: 31–37.
- Maj, J., Dziedzicka-Wasylewska, M., Rogoz, R., Rogoz, Z. and Skuza, G. (1996) Antidepressant drugs given repeatedly change the binding of the dopamine D₂ receptor agonist, [³H]N-0437, to dopamine D₂ receptors in the rat brain. *Eur. J. Pharmacol.*, 304: 49–54.
- Maj, J., Rogoi, Z., Margas, W., Kata, M. and Dziedzicka-Wasylewska, M. (2000) The effect of repeated treatment with pramipexole on the central dopamine D₃ system. *J. Neural Transm.*, 107: 1369–1379.
- Martin, K.F., Phillips, I., Cheetham, S.C. and Heal, D.J. (1995) Dopamine D₂ receptors: a potential pharmacological target for nomifensine and tranlycypromine but not other antidepressant treatments. *Pharmacol. Biochem. Behav.*, 51: 565–569.
- Meyer, J.H., Kruger, S., Wilson, A.A., Christensen, B.K., Goulding, V.S., Schaffer, A., Minifie, C., Houle, S., Hussey, D. and Kennedy, S.H. (2001) Lower dopamine transporter binding potential in striatum during depression. *Neuroreport*, 12: 4121–4125.
- Muscat, R., Papp, M. and Willner, P. (1992) Antidepressant-like effects of dopamine agonists in an animal model of depression. *Biol. Psychiatry*, 31: 937–946.
- Muscat, R., Sampson, D. and Willner, P. (1990) Dopaminergic mechanism of imipramine action in an animal model of depression. *Biol. Psychiatry*, 28: 223–230.

- Nakayama, K. (2002) Effect of paroxetine on extracellular serotonin and dopamine levels in the prefrontal cortex. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 365: 102–105.
- Nakayama, K., Sakurai, T. and Katsu, H. (2004) Mirtazapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. *Brain Res. Bull.*, 63: 237–241.
- Nestler, E.J. and Duman, R.S. (1995) Intracellular messenger pathways as mediators of neural plasticity. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress, Intracellular Messenger Pathways as Mediators of Neural Plasticity*. Raven Press, New York, pp. 695–704.
- Ordway, G.A. and Mann, J.J. (2002) Neurocircuitry of mood disorders. In: Davis K.L., Coyle J.T. and Nemeroff C. (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress, Neurocircuitry of Mood Disorders*. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 1051–1064.
- Overstreet, D.H. (1993) The Flinders sensitive line rats: a genetic animal model of depression. *Neurosci. Biobehav. Rev.*, 17: 51–68.
- Papp, M., Klimek, V. and Willner, P. (1994) Parallel changes in dopamine D₂ receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine. *Psychopharmacology (Berl.)*, 115: 441–446.
- Pare, C.M.B. (1969) 5-hydroxytryptamine, noradrenaline, and dopamine in brainstem, hypothalamus, and caudate nucleus of controls and of patients committing suicide by coal-gas poisoning. *Lancet*, 2: p. 133.
- Parsey, R.V., Oquendo, M.A., Zea-Ponce, Y., Rodenhiser, J., Kegeles, L.S., Prata, M., Cooper, T.B., Van Heertum, R., Mann, J.J. and Laruelle, M. (2001) Dopamine D₂ receptor availability and amphetamine-induced dopamine release in unipolar depression. *Biol. Psychiatry*, 50: 313–322.
- Parsons, L.H. and Justice, J.B., Jr. (1993) Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by *in vivo* microdialysis. *Brain Res.*, 606: 195–199.
- Perugi, G., Toni, C., Ruffolo, G., Frare, F. and Akiskal, H. (2001) Adjunctive dopamine agonists in treatment-resistant bipolar II depression: an open case series. *Pharmacopsychiatry*, 34: 137–141.
- Post, R.M., Gerner, R.H., Carman, J.S., Gillin, J.C., Jimerson, D.C., Goodwin, F.K. and Bunney, W.E., Jr. (1978) Effects of a dopamine agonist pibedil in depressed patients: relationship of pretreatment homovanillic acid to antidepressant response. *Arch. Gen. Psychiatry*, 35: 609–615.
- Pruessner, J.C., Champagne, F., Meaney, M.J. and Dagher, A. (2004) Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [¹¹C]raclopride. *J. Neurosci.*, 24: 2825–2831.
- Reddy, P.L., Khanna, S., Subhash, M.N., Channabasavanna, S.M. and Rao, B.S. (1992) CSF amine metabolites in depression. *Biol. Psychiatry*, 31: 112–118.
- Rogoz, R. and Dziedzicka-Wasylewska, M. (1999) Effects of antidepressant drugs on the dopamine D₂/D₃ receptors in the rat brain differentiated by agonist and antagonist binding — an autoradiographic analysis. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 359: 178–186.
- Sanchez, C. and Hyttel, J. (1999) Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell. Mol. Neurobiol.*, 19: 467–489.
- Sarre, S., Yuan, H., Jonkers, N., Van Hemelrijck, A., Ebinger, G. and Michotte, Y. (2004) *In vivo* characterization of somatodendritic dopamine release in the substantia nigra of 6-hydroxydopamine-lesioned rats. *J. Neurochem.*, 90: 29–39.
- Schultz, W. (1998) Predictive reward signal of dopamine neurons. *J. Neurophysiol.*, 80: 1–27.
- Schultz, W., Dayan, P. and Montague, P.R. (1997) A neural substrate of prediction and reward. *Science*, 275: 1593–1599.
- Schultz, W. and Dickinson, A. (2000) Neuronal coding of prediction errors. *Annu. Rev. Neurosci.*, 23: 473–500.
- Self, D.W. and Nestler, E.J. (1995) Molecular mechanisms of drug reinforcement and addiction. *Annu. Rev. Neurosci.*, 18: 463–495.
- Serretti, A., Cristina, S., Lilli, R., Cusin, C., Lattuada, E., Lorenzi, C., Corradi, B., Grieco, G., Costa, A., Santorelli, F., Barale, F., Nappi, G. and Smeraldi, E. (2002) Family-based association study of 5-HTTLPR, TPH, MAO-A, and DRD4 polymorphisms in mood disorders. *Am. J. Med. Genet.*, 114: 361–369.
- Shopsin, B. and Gershon, S. (1978) Dopamine receptor stimulation in the treatment of depression: pibedil (ET-495). *Neuropsychobiology*, 4: 1–14.
- Stahl, S.M. (2000) *Essential Psychopharmacology: Neuroscientific Basis and Practical Application*. Cambridge University Press, Cambridge, UK.
- Steinmetz, P.N., Manwani, A., Koch, C., London, M. and Segev, I. (2000) Subthreshold voltage noise due to channel fluctuations in active neuronal membranes. *J. Comput. Neurosci.*, 9: 133–148.
- Tanda, G., Carboni, E., Frau, R. and Di Chiara, G. (1994) Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology (Berl.)*, 115: 285–288.
- Theohar, C., Fischer-Cornelissen, K., Brosch, H., Fischer, E.K. and Petrovic, D. (1982) A comparative, multicenter trial between bromocriptine and amitriptyline in the treatment of endogenous depression. *Arzneimittelforschung*, 32: 783–787.
- Vale, S., Espejel, M.A. and Dominguez, J.C. (1971) Amantadine in depression. *Lancet*, 2: p. 437.
- Wahrens, J. and Gerlach, J. (1981) Bromocriptine and imipramine in endogenous depression: a double-blind controlled trial in out-patients. *J. Affect. Disord.*, 3: 193–202.
- Weikop, P., Kehr, J. and Scheel-Kruger, J. (2004) The role of alpha₁- and alpha₂-adrenoreceptors on venlafaxine-induced elevation of extracellular serotonin, noradrenaline and dopamine levels in the rat prefrontal cortex and hippocampus. *J. Psychopharmacol.*, 18: 395–403.
- Willner, P., Hale, A.S. and Argyropoulos, S. (2005) Dopaminergic mechanism of antidepressant action in depressed patients. *J. Affect. Disord.*, 86: 37–45.
- Wise, R.A. (1996) Neurobiology of addiction. *Curr. Opin. Neurobiol.*, 6: 243–251.

- Yadid, G., Nakash, R., Deri, I., Tamar, G., Kinor, N., Gispan, I. and Zangen, A. (2000a) Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Prog. Neurobiol.*, 62: 353–378.
- Yadid, G., Zangen, A., Dmitrochenko, A., Overstreet, D.H. and Zohar, J. (2000b) Screening for new antidepressants with fast onset and long-lasting action. *Drug Dev. Res.*, 50: 392–399.
- Zangen, A., Nakash, R., Overstreet, D.H. and Yadid, G. (2001) Association between depressive behavior and absence of serotonin–dopamine interaction in the nucleus accumbens. *Psychopharmacology (Berl.)*, 155: 434–439.
- Zangen, A., Nakash, R. and Yadid, G. (1999a) Serotonin-mediated increases in the extracellular levels of beta-endorphin in the arcuate nucleus and nucleus accumbens: a microdialysis study. *J. Neurochem.*, 73: 2569–2574.
- Zangen, A., Overstreet, D.H. and Yadid, G. (1997) High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. *J. Neurochem.*, 69: 2477–2483.
- Zangen, A., Overstreet, D.H. and Yadid, G. (1999b) Increased catecholamine levels in specific brain regions of a rat model of depression: normalization by chronic antidepressant treatment. *Brain Res.*, 824: 243–250.

CHAPTER 14

Physiological and therapeutic relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors for the treatment of depression

Kelly A. Berg¹, John A. Harvey², Umberto Spampinato³ and William P. Clarke^{1,*}

¹*Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78229-3900, USA*

²*Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102-1192, USA*

³*Université Victor Segalen Bordeaux 2, Centre de Recherche Inserm U862, Institut Francois Magendie, 146, rue Léo Saignat, B.P. 59, 33077 Bordeaux Cedex, France*

Abstract: Serotonin_{2A} (5-HT_{2A}) and 5-HT_{2C} receptors are highly homologous members of the serotonin₂ family of 7-transmembrane-spanning (7-TMS) receptors. Both of these receptor subtypes have been implicated in the aetiology and/or treatment of affective disorders such as anxiety and depression. Regulation of dopaminergic neurotransmission by 5-HT_{2A} and 5-HT_{2C} receptor systems has been well established. In general, agonist activation of 5-HT_{2A} receptors can facilitate stimulated dopamine (DA) release, whereas 5-HT_{2C} agonists inhibit dopaminergic neural activity and DA release under both basal and activated conditions. However, recent experimental evidence suggests that 5-HT_{2A} and 5-HT_{2C} receptors can be constitutively active (agonist-independent activity) *in vivo*. Alterations in the constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptor systems could be involved in the mechanisms underlying anxiety and depression or exploited for therapeutic benefit. Consequently, drugs with inverse agonist properties may have more activity *in vivo* to regulate DA neurotransmission than that afforded by simple competitive antagonism.

Keywords: inverse agonism; agonist-independent receptor activity; dopamine; dopaminergic neurotransmission; signal transduction

Introduction

Serotonin_{2A} (5-HT_{2A}) and 5-HT_{2C} receptors are members of the super-family of 7-transmembrane-spanning (7-TMS) receptors, also known as G protein-coupled receptors. Grouped within the

subclass of 5-HT₂ receptors, these receptors share a high degree of amino acid sequence homology ($\approx 80\%$ in the transmembrane regions). As might therefore be expected, the pharmacological characteristics of these receptors are quite similar, with relatively few selective ligands available. In addition, some of the signalling cascades that are activated by these receptors are the same [e.g. phospholipase C (PLC) and PLA₂]. In spite of these similarities, there are also significant differences

*Corresponding author. Tel.: +1-210-567-4171;
Fax: +1-210-567-6952; E-mail: clarkew@uthscsa.edu

between the 5-HT_{2A} and 5-HT_{2C} receptor systems (Roth et al., 1986, 1998; Berg et al., 1994, 2001b; Grotewiel and Sanders-Bush, 1999; Boulougouris et al., 2008; Robinson et al., 2008). 5-HT_{2A} and 5-HT_{2C} receptors are widely distributed throughout the brain, notably present in corticolimbic areas, such as the amygdala, hippocampus, frontal cortex, ventral tegmental area (VTA), nucleus accumbens (NAc) and hypothalamus. Consequently, it is not surprising that these receptors have been implicated in the aetiology of various affective disorders, including depression and anxiety (Wood, 2003; Leysen, 2004; Millan, 2005, 2006a).

5-HT_{2C} receptors and depression and anxiety

There is a large literature that points to a role of the 5-HT_{2C} receptor in depression (for reviews see Wood, 2003; Giorgetti and Tecott, 2004; Esposito, 2006; Millan, 2005, 2006a). 5-HT_{2C} receptor density and responsiveness is enhanced in experimental models of depression and in humans (Moreau et al., 1993; Fone et al., 1996; Iwamoto and Kato, 2003; Yang et al., 2004). Various treatments for depression [including selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, etc.], but not all, reduce 5-HT_{2C} receptor function (see Millan, 2005). Although not rigorously studied, selective 5-HT_{2C} receptor antagonists appear to have antidepressant-like activity in some animal models (Bakish et al., 1993). 5-HT_{2C} receptor antagonists/inverse agonists increase dopaminergic neurotransmission (see below), an effect that would be expected to have a beneficial effect on mood and cognitive function, which are reduced in depression. Effects of SSRIs can also be potentiated by selective 5-HT_{2C} antagonists (Cryan and Lucki, 2000; Cremers et al., 2004) and several antidepressant drugs have antagonist action at 5-HT_{2C} receptors (Jenck et al., 1994; Palvimäki et al., 1996; Ni and Miledi, 1997; Giorgetti and Tecott, 2004).

Interestingly, there are also data suggesting that 5-HT_{2C} agonists may have antidepressant activity. Decreases in immobility time and increases in swimming behaviour in rats in the forced swim test occur in response to 5-HT_{2C} agonists in a manner

comparable to those elicited by the SSRI fluoxetine (Cryan and Lucki, 2000). In addition, compounds with 5-HT_{2C} agonist properties have been shown to be active in various models for antidepressant activity (Moreau et al., 1996; Martin et al., 1998; Mitchell and Redfern, 2005). Recently a new compound, WAY-163909, which has agonist properties (for calcium mobilization and decreasing food intake) and very high affinity for the 5-HT_{2C} receptor (Dunlop et al., 2005), has been shown to be active in a variety of models for antidepressant activity (Rosenzweig-Lipson et al., 2008). The argument that the antidepressant-like effects of 5-HT_{2C} agonists are due to desensitization of the 5-HT_{2C} receptor system was deemed unlikely in a study by Rosenzweig-Lipson et al. (2007) because acute administration of WAY-163909 was as effective as chronic administration and effects of WAY-163909 could be blocked by 5-HT_{2C} receptor antagonists. Although the desensitization of 5-HT_{2C} receptor signalling (Berg et al., 2001b; Stout et al., 2002) and receptor phosphorylation (Westphal et al., 1995) can occur rapidly (<30 min), at the present time, it is difficult to reconcile the evidence for antidepressant effects of both 5-HT_{2C} agonists and antagonists.

Acute activation of 5-HT_{2C} receptors, with agonists such as mCPP and MK-212, results in feelings of anxiety and panic in humans (Mueller et al., 1985; Charney et al., 1987; Lowy and Meltzer, 1988; Benjamin et al., 1990, 1999; Kahn and Wetzler, 1991; Klein et al., 1991; Southwick et al., 1997; Gatch, 2003) and induces anxiogenic-like behaviour in animals (Kennett et al., 1989; Griebel et al., 1991; Bilkei-Gorzo et al., 1998; Bagdy et al., 2001; Jones et al., 2002; Martin et al., 2002; Campbell and Merchant, 2003; de Mello Cruz et al., 2005; Millan, 2006b; Cornelio and Nunes-de-Souza, 2007; Hackler et al., 2007). 5-HT_{2C} antagonists, on the other hand, can block the anxiogenic-like behaviour induced by 5-HT_{2C} agonists (Kennett et al., 1989; Bagdy et al., 2001; Campbell and Merchant, 2003; de Mello Cruz et al., 2005; Cornelio and Nunes-de-Souza, 2007; Hackler et al., 2007) and are also anxiolytic when administered alone (Kennett et al., 1995, 1997; Wood et al., 2001; Wood, 2003; Hackler et al., 2007). Interestingly, anxiogenic behaviour induced by acute

administration of SSRI antidepressants (e.g. fluoxetine) can be blocked by 5-HT_{2C} antagonists (Bagdy et al., 2001) and with prolonged SSRI administration, 5-HT_{2C} receptors down-regulate with a time course similar to that associated with improvement of clinical symptoms (Bristow et al., 2000).

5-HT_{2C} receptor and mRNA editing

The 5-HT_{2C} receptor is, to date, the only 7-TMS receptor found to undergo the post-transcriptional process of mRNA editing, which generates unique isoforms of proteins in a cell- and/or tissue-specific manner (Simpson and Emeson, 1996; Smith et al., 1997). mRNA transcripts of the rat and human 5-HT_{2C} receptors undergo adenosine-to-inosine editing events at five sites that encompass amino acids 156–160 within the putative second intracellular domain of the encoded human receptor, resulting in the production of 14 5-HT_{2C} receptor isoforms (Burns et al., 1997; Niswender et al., 1999). In human brain, the non-edited receptor contains the amino acids isoleucine, asparagine and isoleucine (INI) at positions 156, 158 and 160, respectively, while two of the principal edited isoforms expressed have valine, serine and valine (VSV) or valine, glycine and valine (VGV), corresponding to these amino acid positions (156, 158 and 160, respectively). Affinity of 5-HT (and therefore the potency to activate PLC and PLA₂) is reduced for VSV or VGV receptor isoforms in comparison with the non-edited INI receptor (Burns et al., 1997; Fitzgerald et al., 1999; Niswender et al., 1999; Berg et al., 2001a). RNA-edited receptors also have altered signal transduction profiles (Price and Sanders-Bush, 2000; Price et al., 2001; Berg et al., 2001a; McGrew et al., 2004), which may not be surprising since editing changes amino acids in the second intracellular domain, a region important for G protein coupling and signal transduction (Bourne, 1997; Gudermann et al., 1997; Wess, 1998).

Recent studies suggest that RNA editing of 5-HT_{2C} receptor transcripts may be altered in depression. Several human studies have compared prefrontal cortical RNA-editing patterns in patients with psychiatric disorders with those in

normal controls and produced conflicting results. For example, in brains of suicide patients diagnosed with depression, RNA editing was reported to be enhanced (Gurevich et al., 2002), resulting in a lower frequency of the non-edited (5-HT_{2C}-INI) and partially edited (5-HT_{2C}-VNI) receptor mRNA transcripts, compared to control patients. A slightly different result was reported by Niswender et al. (2001), who found that frequency of RNA transcripts encoding the VNI isoform was increased in suicides compared to non-suicides. Consistent with Niswender et al. (2001), Iwamoto and Kato (2003) reported a trend for increased 5-HT_{2C}-VNI transcripts in suicides.

In animal models, the pattern of RNA editing in brain can be altered by various stressors and drug treatments. Stress induced by the forced swim test in mice leads to changes in RNA editing that are reversed by treatment with the antidepressant fluoxetine (Englander et al., 2005). Learned helplessness in rats is associated with a slight increase in RNA-editing efficiency, which is reversed by treatment with the antidepressants fluoxetine and imipramine (Iwamoto et al., 2005). Early life stress robustly increases RNA editing in adult mice, which is reduced by treatment with fluoxetine during adolescence (Bhansali et al., 2007).

5-HT_{2A} receptor and depression

Early observations that chronic treatment of rats with antidepressant drugs produced a decrease in the [³H]-spiperone (a non-selective 5-HT_{2A} receptor antagonist) binding density in cortex (Peroutka and Snyder, 1980; Kellar et al., 1981) led to the hypothesis that 5-HT_{2A} receptors were involved in the aetiology of depression. Consistent with this hypothesis, prolonged tricyclic antidepressant treatment reduces 5-HT_{2A} binding sites in rat cortex (Lafaille et al., 1991; Moreau et al., 2001). 5-HT_{2A} receptors internalize following prolonged exposure to agonists and, paradoxically, some antagonists (Willins et al., 1999; Van Oekelen et al., 2003). Some SSRI antidepressants have 5-HT_{2A} antagonist properties (Leysen, 2004) and increase synaptic levels of 5-HT. Consequently, it is expected that SSRI treatment would result in

decreased 5-HT_{2A} receptor density (Van Oekelen et al., 2003). Furthermore, treatment of rats with the selective 5-HT_{2A} antagonist, M100907, produces some behavioural responses characteristic of antidepressant drugs (Marek et al., 2005).

Results of studies on 5-HT_{2A} binding with human tissue have been equivocal, with some studies reporting increased, decreased or no change in 5-HT_{2A} binding in post-mortem samples from suicide victims, depending upon the brain region and diagnosis (see Stockmeier, 2003 for review). Antagonism of 5-HT_{2A} receptors appears to increase the therapeutic effectiveness of SSRI and other antidepressant drugs (Marek et al., 2003), suggesting that the activation of 5-HT_{2A} receptors by increased 5-HT neurotransmission can oppose the therapeutic effects of antidepressant drug therapy.

5-HT_{2A} receptors may play an important role in the cognitive dysfunctions that are part of the depression syndrome. Depressed individuals have deficits in a variety of cognitive functions, such as memory, executive functions, attention and information-processing speed. Regulation of 5-HT_{2A} receptor activity has been shown to influence cognitive functions; however, the effect of 5-HT_{2A} ligands is complex and appears to depend upon the system studied and the experimental paradigm used. 5-HT_{2A} agonism enhances glutamatergic neurotransmission in cortical areas (Aghajanian and Marek, 2000), which would be expected to improve cognition (Buchanan et al., 2007; Gray and Roth, 2007). Accordingly, 5-HT_{2A} agonists increase, and inverse agonists decrease, the acquisition of an associative-learning task in rabbits (Harvey, 2003; Berg et al., 2005). In addition, infusion of a selective 5-HT_{2A} antagonist into the NAc impairs performance in a spatial reversal-learning task (Boulougouris et al., 2008), suggesting that activation of 5-HT_{2A} receptors may facilitate cognitive flexibility. However, 5-HT_{2A} agonists impair, and antagonists enhance, working memory in monkeys (Williams et al., 2002; Terry et al., 2005). In addition, 5-HT_{2A} antagonists applied directly to the NAc reduce impulsivity (Robinson et al., 2008), suggesting that the antagonism of 5-HT_{2A} receptors may help improve attention deficits in depression.

Antagonism/inverse agonism at 5-HT_{2A} receptors, which results from treatment with atypical antipsychotics, may also improve cognitive performance in schizophrenic patients (Roth et al., 2004; Gray and Roth, 2007). Given these disparate findings, it seems clear that more research is needed to unravel the role of 5-HT_{2A} receptors and cognitive functions.

5-HT_{2A} and 5-HT_{2C} receptor-mediated regulation of dopaminergic neurotransmission

Regulation of dopaminergic neurotransmission by 5-HT_{2A} and 5-HT_{2C} receptors is well established (for reviews, see Esposito, 2006; Bubar and Cunningham, 2007). Both receptors are found in the substantia nigra and VTA (where dopaminergic cell bodies are located) and in forebrain target areas of dopaminergic neurons (Eberle-Wang et al., 1997; Mengod et al., 1997; Barnes and Sharp, 1999). In general, in the mesocorticolimbic system, activation of 5-HT_{2A} receptors can facilitate stimulated, but not basal, dopamine (DA) release (Schmidt et al., 1992; Prisco et al., 1994; Schmidt and Fadaye, 1996; Porras et al., 2002), whereas 5-HT_{2C} receptor activation inhibits dopaminergic neuronal activity and DA release (Gobert et al., 2000; De Deurwaerdere and Spampinato, 2001; Di Giovanni et al., 2001; De Deurwaerdere et al., 2004), likely by activating GABAergic inhibitory neurons (Invernizzi et al., 2007). Although serotonergic and noradrenergic neurotransmission have held centre stage as substrates underlying affective disorders, such as depression and anxiety, dysfunction of DA systems is also involved in the pathophysiology of depression, especially with respect to its hedonic and motivational aspects (Esposito, 2006; Dunlop and Nemeroff, 2007; Friedman et al., 2007). Consequently, serotonergic regulation of dopaminergic neurotransmission via 5-HT_{2A} and 5-HT_{2C} receptors may be important in regulating the affective state and the response to antidepressant and anxiolytic drugs.

As mentioned above, 5-HT_{2C} receptor agonists decrease basal dopaminergic cell firing and DA release. The opposite effect occurs with the administration of 5-HT_{2C} receptor antagonists,

which increase dopaminergic neurotransmission (Gobert et al., 2000; De Deurwaerdere and Spampinato, 2001). This enhancement of dopaminergic activity was first attributed to blockade of endogenous serotonin (Di Giovanni et al., 1999); however, it has recently been established that ligand-independent (constitutive) 5-HT_{2C} receptor activity maintains a tonic inhibitory tone on dopaminergic transmission (De Deurwaerdere et al., 2004; Navailles et al., 2006b). Ligands, previously defined as antagonists, with inverse agonist properties reduce the constitutive activity of the 5-HT_{2C} receptor and increase DA release.

Regulation of dopaminergic neurotransmission by constitutive receptor activity has important implications for understanding the pathophysiology of depression and anxiety as well as for drug development. The remainder of this chapter will present some background information on constitutive receptor activity and inverse agonism and then discuss the evidence of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors.

Constitutive receptor activity

Historically, receptors in a population were thought to exist in a quiescent state, until activated by a ligand that possessed both affinity (ability to bind to receptors) and intrinsic efficacy (ability to activate receptors). Receptor activation involved induction of a change in conformation that would allow the receptor to regulate the activity of various cellular signal transduction cascades. Within this framework, two general classes of ligands existed. Ligands with zero intrinsic efficacy were known as antagonists, which could bind but not activate the receptor. All other ligands possessed some degree of intrinsic efficacy above zero and were known as agonists. Agonists could be subdivided into full agonists (those with large values of intrinsic efficacy, which produce a maximal level of a response) and partial agonists (with lower levels of intrinsic efficacy).

Since the pioneering work of Cerione et al. (1984), with purified β -adrenergic receptor and G α s proteins, and Costa and Herz (1989), with opioid receptors expressed natively in intact cells,

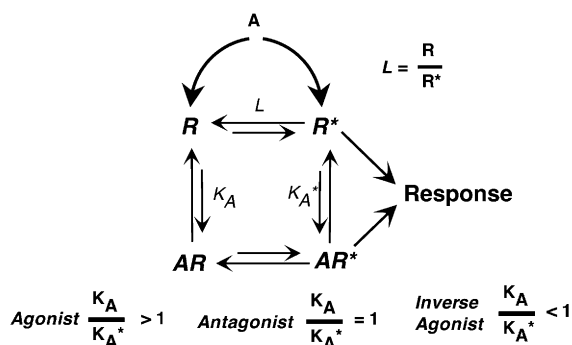


Fig. 1. Two-state model of receptor activation, derived from the work of Leff (1995). Ligands (A) bind to R and R*, with equilibrium dissociation constants K_A and K_{A^*} , respectively. L is an isomerization constant (sometimes referred to as an allosteric constant, see Christopoulos and Kenakin, 2002) that defines the probability of finding a receptor in the active conformation. Agonists (ligands with $K_A/K_{A^*} > 1$) enrich the R* population and thus increase response. Inverse agonists ($K_A/K_{A^*} < 1$) enrich the R population and thus deplete R* and reduce basal response. Antagonists ($K_A = K_{A^*}$) do not alter the proportions of R vs. R* and thus do not change basal response, but because they occupy the receptor population, they can antagonize the action of both agonists and inverse agonists.

it has been established that receptors can be active (activate cellular signal transduction pathways) in the absence of an activating ligand. Current ideas of receptor function are now based on multi-state models in which receptors in a population exist in equilibrium with an inactive (R) and one or more active conformations (R*). The simplest of these multi-state models is a two-state model (Leff, 1995), which is presented in Fig. 1. It is now generally accepted that most, if not all, receptors possess some level of constitutive receptor activity.

In contrast to the traditional model, where agonists induce a conformational change in a receptor to confer activity, in the two-state model, the active receptor state already exists to a degree specified by the allosteric constant (L). Agonists select the active conformation by virtue of having higher affinity for this state and thereby enrich the population of active receptors, leading to increased level of response. Antagonists, with equal affinity for both conformations, do not alter the proportion of active to inactive receptors and do not change the ongoing level of response. Intrinsic efficacy, therefore, can be defined as the ratio of

affinity constants for the inactive vs. the active receptors (K_A/K_{A^*}). The greater the difference in affinity between the inactive and active conformations, the greater the intrinsic efficacy of the ligand. The K_A/K_{A^*} ratio for antagonists is 1.

The existence of multiple receptor conformations and constitutive receptor activity allows for another pharmacological class of ligands. In addition to agonists, with K_A/K_{A^*} ratios >1 , and antagonists, with K_A/K_{A^*} ratios equal to 1, ligands can also have K_A/K_{A^*} ratios <1 . Such ligands are known as inverse agonists. By having greater affinity for the inactive conformation, inverse agonists enrich this population at the expense of the active receptor population. The reduced quantity of active receptors leads to a reduction in the ongoing level of response. The existence of inverse agonists significantly enriches the pharmacological treasure chest and permits manipulation of receptor activity in two different directions.

Although the two-state model allows for the existence of constitutive receptor activity and inverse agonism, it cannot accommodate all of the experimental findings of receptor function. For example, it is known that different agonists acting at the same receptor subtype can differentially regulate the activity of each of several signalling cascades in cells (Kenakin, 1995; Berg and Clarke, 2006; Urban et al., 2007). For the 5-HT_{2C} receptor, agonists such as 2,5-dimethoxy-4-iodophenylisopropylamine (DOI), lysergic acid diethylamide (LSD) and bufotenin have greater relative efficacy for activation of the PLC pathway than the PLA₂ pathway, whereas other agonists [e.g. quipazine, 3-trifluoromethylphenyl-piperazine (TFMPP)] acting at the same receptor in the same cells have greater activity towards the PLA₂ than the PLC response (Berg et al., 1998, 2001a; Moya et al., 2007). This response-dependent behaviour of agonists has been called agonist-directed trafficking of receptor stimulus, biased agonism, stimulus trafficking and functional selectivity.

To accommodate such agonist behaviour, Leff et al. (1997) developed a three-state model (Fig. 2) where receptors are proposed to exist in an inactive conformation (R) in equilibrium with two active conformations (R*, R**), each of which can regulate the activity of different cellular effector

pathways. Ligands can differentially enrich or deplete these conformations. However, it is quite likely that receptors are not restricted to just three states but rather can adopt a large variety of conformations. In this regard, Kenakin (2002) has proposed the ensemble theory, which suggests that groups of receptor conformations may act in a similar manner.

Multi-state models allow for different levels of constitutive receptor activity towards different responses coupled to a receptor. Consequently, the efficacy of inverse agonists, like that of agonists, can be response dependent. Furthermore, it is possible for a ligand to enrich an active conformation (R*) at the expense of depleting a different active conformation (R**). Such ligands would be agonists for one response and inverse agonists for the other response at the same time. This type of ligand behaviour has been observed by several groups (Ganguli et al., 1998; Pauwels et al., 2002; Newman-Tancredi et al., 2003; De Deurwaerdere et al., 2004; Lane et al., 2007) and the ligands that act in this way have been termed protean agonists (Kenakin, 2001; Chidiac, 2002; Perez and Karnik, 2005; Neubig, 2007).

5-HT_{2A} and 5-HT_{2C} constitutive receptor activity in vitro

In vitro, both 5-HT_{2A} and 5-HT_{2C} receptors have been shown to exhibit constitutive activity. In general, it appears that the constitutive activity of the 5-HT_{2C} receptor towards the PLC pathway is higher than that of the 5-HT_{2A} receptor. Agonist-independent activity towards PLC is readily observed for the 5-HT_{2C} receptor (Barker et al., 1994; Westphal et al., 1995; Niswender et al., 1999; Herrick-Davis et al., 1999, 2000; Berg et al., 1999, 2006); however, there are no reports of constitutive 5-HT_{2A} receptor activity to PLC in vitro without either mutation of the receptor (Egan et al., 1998; Shapiro et al., 2002; Teitler et al., 2002) or over-expression of G proteins (Weiner et al., 2001) to enhance constitutive activity. Although caution must be used when comparing the constitutive activity of receptor systems in different cells/tissues since cell phenotype can influence the expression of

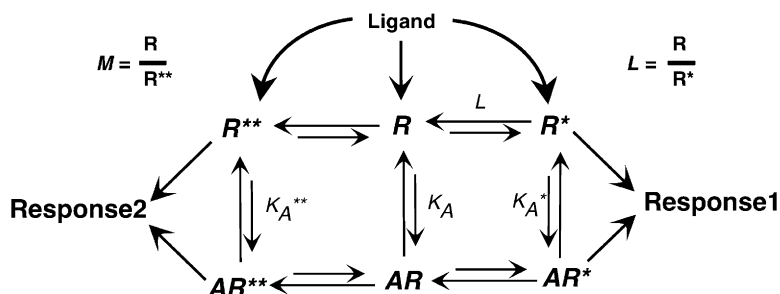


Fig. 2. Three-state model of receptor function. The receptor population consists of an inactive receptor conformation (R) in equilibrium with two active receptor conformations (R^* and R^{**}). Each active conformation can differentially activate effector mechanisms leading to response1 or response2 in the absence of an agonist. Two isomerization constants (L and M) define the propensity of the receptor to adopt an active conformation in the absence of a ligand. Agonists can differentially stabilize R^* vs. R^{**} , depending upon the value of the equilibrium dissociation constants K_{A^*} and $K_{A^{**}}$ relative to K_A . Inverse agonists can also have differential effects on response1 vs. response2, depending upon the relative values of L and M . Adapted with permission from Leff et al. (1997) and Berg et al. (1998).

constitutive receptor activity, the 5-HT_{2C} receptor displays approximately 10-fold higher constitutive activity than the 5-HT_{2A} receptor even when the 5-HT_{2A} and 5-HT_{2C} receptors are expressed in the same cell background and the same response is measured as a readout (PLC) (Teitler et al., 2002; Berg et al., unpublished observations).

As mentioned above, multi-state receptor models predict that, like ligand-dependent activity, constitutive receptor activity should be dependent upon the response measured. We, and others, have shown that the 5-HT_{2C} receptor displays a relatively high degree of constitutive activity towards PLC (Barker et al., 1994; Herrick-Davis et al., 1999; Niswender et al., 1999; Berg et al., 1999, 2006); however, constitutive activity towards the PLA₂ pathway is considerably weaker. Accordingly, inverse agonist efficacy is also greater for the PLC response vs. the PLA₂ response (Berg et al., 1999). Although constitutive 5-HT_{2A} signalling to PLC appears to be very weak, Shapiro et al. (2002) and Weiner et al. (2001) report that constitutive activity of the 5-HT_{2A} receptor is stronger for a reporter gene assay.

Further evidence of the response dependence of constitutive activity of 5-HT_{2C} receptors comes from studies of desensitization which, as for agonist-stimulated receptors, can result from constitutive receptor activity (Berg et al., 1999; Wilbanks et al., 2002). Desensitization (a time-dependent loss of responsiveness to receptor

activation) can occur through a variety of mechanisms that include, generally, uncoupling of the receptor from an effector mechanism and/or internalization and down-regulation of the receptor protein from the cell surface (Gainetdinov et al., 2004). Prolonged treatment with an inverse agonist can reduce constitutive activation of desensitization mechanisms, which can be visualized as an increase in responsiveness to both ligand-dependent and ligand-independent receptor activity towards a signalling pathway or by increased cell surface receptor levels, or both. Reduction in constitutive 5-HT_{2C} receptor activity by prolonged treatment with inverse agonists in CHO cells heterologously expressing 5-HT_{2C} receptors (INI) increases receptor-mediated PLC-inositol phosphate (IP), but not PLA₂-arachidonic acid (AA), signalling (Berg et al., 1999), suggesting that the 5-HT_{2C}-PLC effector pathway is selectively, constitutively desensitized in these cells. This constitutive desensitization is especially sensitive to constitutive receptor activity, occurring at low levels of 5-HT_{2C} receptor expression (250 fmol/mg protein). At this low level of expression, ligands that behave as simple competitive antagonists towards 5-HT_{2C} receptor-mediated activation of PLC and PLA₂ behave as strong inverse agonists for the desensitization response. The mechanism for the constitutive desensitization of PLC signalling involves decreased levels of Gαq/11; inverse agonist treatment results in increased Gαq/11 levels (Berg et al., 1999).

Interestingly, the increased $G\alpha_q/11$ levels in response to 5-HT_{2C} inverse agonist treatment promote an increase in the responsiveness of a purinergic receptor that is coupled to PLC and is endogenously expressed in the cells (heterologous sensitization). This result suggests that constitutive activity (and inverse agonist action) of one receptor subtype can influence the function of heterologous receptor systems.

In general, it appears that constitutive activity of RNA-edited 5-HT_{2C} receptor isoforms towards PLC is reduced compared with that of the non-edited receptor. Although it has been suggested that the reason for the lack of constitutive activity towards PLC is due to reduced capacity of the edited isoforms to couple to G proteins (Fitzgerald et al., 1999; Herrick-Davis et al., 1999; Niswender et al., 1999; Wang et al., 2000), it is not clear whether the reduced coupling is due to a reduction in the number of receptors in an active conformation (reduced isomerization) or a reduced capacity of active receptors to couple to G proteins. The latter possibility is suggested since the second intracellular loop is known to play a role in G protein coupling (Bourne, 1997; Gudermann et al., 1997; Wess, 1998). However, there is a report (Niswender et al., 1999) suggesting that the effect is not due to changes in G protein coupling since there was no difference in the maximal response to 5-HT between the edited and the non-edited isoforms. Also, RNA editing reduces the binding affinity of agonists (Fitzgerald et al., 1999; Niswender et al., 1999), as would be expected if the capacity of the receptor to adopt an active conformation were reduced (Kenakin, 2001).

5-HT_{2A} constitutive receptor activity in vivo

Although the constitutive activity of the 5-HT_{2A} receptor studied in vitro appears to be weak (see above), a series of studies by Harvey et al. reveal that the 5-HT_{2A} receptor can display a significant level of constitutive activity in vivo (Welsh et al., 1998; Harvey et al., 1999, 2004; Romano et al., 2000, 2006; Harvey, 2003; Berg et al., 2005; Dave et al., 2007). The collective work by the Harvey group found that drugs previously characterized as

antagonists did not have a uniform effect on the rate of conditioning of the eyeblink reflex in the rabbit (a model of associative learning). Whereas 5-HT_{2A} agonists such as DOI, LSD, 2,5-dimethoxy-4-methylphenylisopropylamine (DOM), 3,4-methylenedioxymphetamine (MDA) and 3,4-methylenedioxymphetamine (MDMA) enhanced the acquisition of the associative-learning task, some putative antagonists (e.g. ketanserin, LY 53857 and BOL), when administered alone, had no effect on learning while others (e.g. mianserin, MDL 100907, MDL 11939, ritanserin and pizotifen) retarded learning (Harvey et al., 2004). This differential efficacy of antagonists on learning is not consistent with simple blockade of the endogenous agonist (5-HT) where the maximal effect of all antagonists should be the same. Differences in the efficacy of antagonists suggest that the drugs have different pharmacological properties, some being antagonists (those with no effect on learning) and some being inverse agonists (those that retarded learning), which reduce constitutive 5-HT_{2A} receptor activity.

Although the differential efficacy of putative antagonists strongly suggests that the 5-HT_{2A} receptor is constitutively active towards associative learning in the rabbit eyeblink model, additional experiments are required to establish this conclusion. Since all ligands have some degree of non-selectivity, it is important to establish that the actions of drugs thought to be inverse agonists are mediated by the target receptor (5-HT_{2A} receptor). Ligand effects that are mediated by the 5-HT_{2A} receptor should be mutually exclusive. Figure 3 shows that ritanserin (inverse agonist, 1 µmol/kg) retarded conditioned response (CR) acquisition and blocked the enhancement of acquisition produced by the agonist LSD (0.03 µmol/kg) (Welsh et al., 1998). Additionally, the antagonist BOL (5.8 µmol/kg) blocked the retardation of learning produced by the inverse agonist mianserin (10 µmol/kg) (Romano et al., 2000). These data indicate that the actions of drugs to both enhance (agonism) and retard (inverse agonism) learning are mediated by the 5-HT_{2A} receptor.

Although the differential efficacy of antagonists suggests that the actions of putative inverse agonists are not due to blockade of the endogenous ligand,

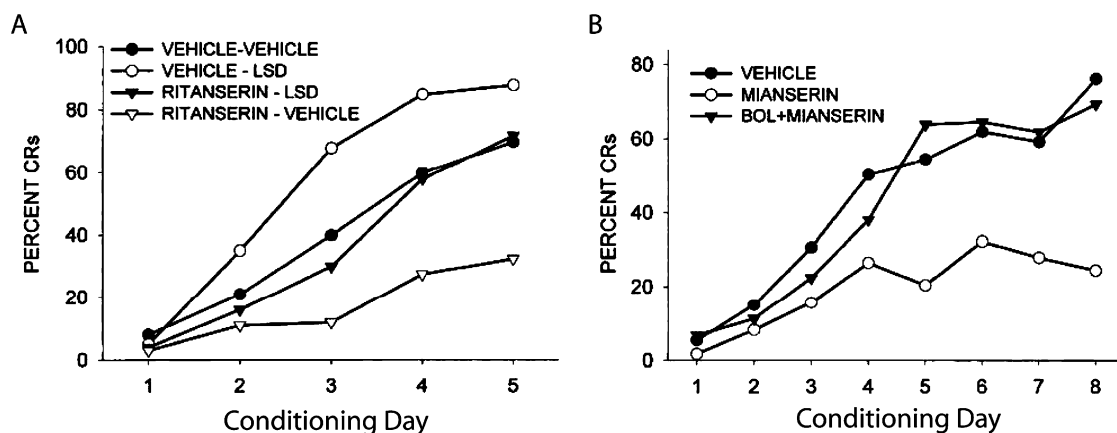


Fig. 3. The effects of 5-HT_{2A} inverse agonists are blocked by 5-HT_{2A} agonists and antagonists. (A) The inverse agonist ritanserin (1 μ mol/kg) retards, and the agonist LSD (0.030 μ mol/kg) enhances, acquisition of a conditioned response (CR, eyeblink) produced by the pairing of a tone (conditioning stimulus) with an air puff (unconditioned response). Ritanserin was injected subcutaneously 60 min and LSD intravenously 20 min prior to each acquisition session. (B) The retardant effects of the inverse agonist mianserin (10 μ mol/kg) on acquisition of the eyeblink response is blocked by the antagonist BOL (5.8 μ mol/kg). Mianserin was injected 1 h and BOL 20 min prior to each conditioning session. Mianserin and BOL were injected subcutaneously. Adapted with permission from Harvey (2003).

it is important to directly test this hypothesis by performing experiments to remove the endogenous agonist. The Harvey group used two approaches to rule out a role for endogenous 5-HT. First, release of 5-HT from serotonergic neurons was reduced by the activation of 5-HT_{1A} somatodendritic autoreceptors with 8-hydroxy-2-(di-n-propylamino)tertraline (8-OH-DPAT) or lisuride. Neither 8-OH-DPAT nor lisuride altered the rate of acquisition of the eyeblink reflex (Welsh et al., 1998), suggesting that endogenous 5-HT did not play a role in learning response. Second, depletion of endogenous 5-HT, using the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), had no effect on 5-HT_{2A} receptor density or learning acquisition. Furthermore, 5-HT depletion did not alter the ability of MDL 11939 to retard learning (Romano et al., 2006). Altogether, these data suggest that endogenous 5-HT does not play a role in learning acquisition in this behavioural model, and that the action of putative antagonists to retard learning is due to reduced constitutive receptor activity as a result of the inverse agonist properties of these ligands.

In some systems, constitutive activity of a receptor leads to constitutive down-regulation of the receptor (MacEwan and Milligan, 1996; Lee et al., 1997; Milligan and Bond, 1997; Stevens

et al., 2000; Wilbanks et al., 2002). Reduction of constitutive receptor activity by treatment with inverse agonists can reduce this constitutive down-regulation, thereby producing an up-regulation of the receptor number on the cell surface. As shown in Fig. 4, chronic treatment (8 days) with 5-HT_{2A} inverse agonists up-regulates 5-HT_{2A} receptor density (Aloyo et al., 2001; Dave et al., 2007) in the rabbit frontal cortex and, as a consequence of higher constitutive receptor activity (due to increased receptor density), increases the rate of learning (Harvey et al., 2004).

Taken together, these data strongly suggest that constitutive 5-HT_{2A} receptor activity in the rabbit plays a role in associative learning. Acute decreases in constitutive activity produced by 5-HT_{2A} inverse agonists retard learning acquisition. Prolonged inverse agonist treatment, which increases constitutive receptor activity by virtue of increasing receptor density, enhances the rate of learning. The differential efficacy of putative antagonists along with the lack of effect of reduced 5-HT release on the rate of learning or on the effect of inverse agonists to retard learning strongly suggests that reduced constitutive activity of the 5-HT_{2A} receptor, not blockade of endogenous 5-HT, is responsible for the actions of inverse agonists in this associative-learning paradigm in

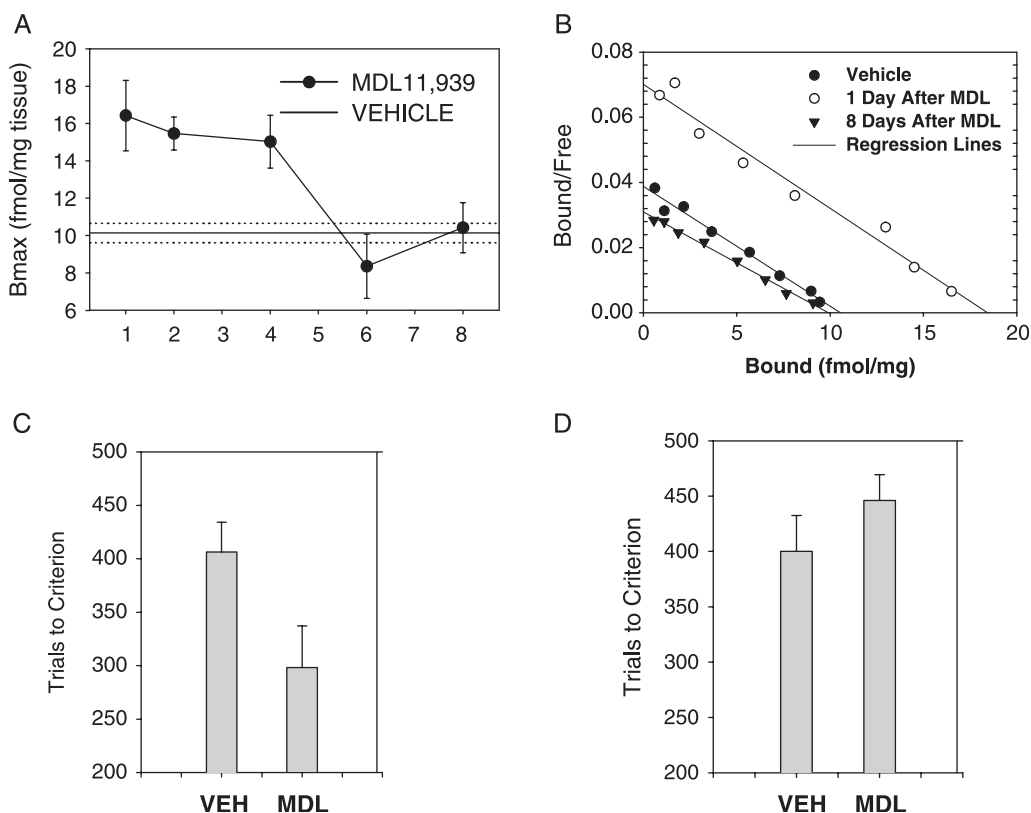


Fig. 4. Prolonged treatment with the 5-HT_{2A} inverse agonist MDL 11939 increases 5-HT_{2A} receptor density in rabbit frontal cortex and enhances acquisition of the eyeblink response. (A) Time course for MDL 11939 induced increases in the density of 5-HT_{2A} receptors in frontal cortex as a function of days after chronic (8-day) treatment with MDL 11939 (10 μ mol/kg; 3.0 mg/kg). The solid horizontal line presents the mean B_{max} for 13 vehicle controls and the dotted lines represent 1 SEM above and below that value. Values for the MDL 11939-treated group are based on three–four animals at each time point. Vertical bars represent \pm 1 SEM. (B) Scatchard analysis of [³H]ketanserin binding to rabbit cortical 5-HT_{2A} receptors for a vehicle control animal and at 1 and 8 days after chronic MDL 11939 treatment. Saturation analyses were performed using eight concentrations of [³H]ketanserin in the presence of 5 mmol/l MgSO₄ (final concentration). (C) and (D) The effect of chronic (8-day) MDL 11939 (10 μ mol/kg; 3.0 mg/kg) administration on acquisition of the conditioned eyeblink response. Animals began acquisition training either 1 day (C) or 8 days (D) after the last injection of MDL 11939 or its vehicle. (C) and (D) give the number of trials required to attain the stringent acquisition criterion of 10 consecutive conditioned responses. Vertical bars represent \pm 1 SEM. Adapted with permission from Harvey et al. (2004).

the rabbit. Given the pronounced cognitive deficits that occur in depression, regulation of constitutive activity of the 5-HT_{2A} receptor may be an important target for pharmacotherapy.

5-HT_{2C} constitutive receptor activity in vivo

Evidence for a role of 5-HT_{2C} constitutive receptor activity in vivo comes from microdialysis studies (De Deurwaerdere et al., 2004) assessing the role of

central 5-HT_{2C} receptors in the tonic inhibitory control of ascending dopamine pathways activity in the rat brain. Systemic administration of purported 5-HT_{2C} antagonists (SB 242084, SB 206553) significantly enhances basal DA release in DA-innervated areas of the rat brain, such as the frontal cortex, the NAc and the striatum (Gobert et al., 2000; De Deurwaerdere and Spampinato, 2001). The magnitude of this effect however differs with different antagonists, SB 206553 being more efficacious than SB 242084 in enhancing basal DA

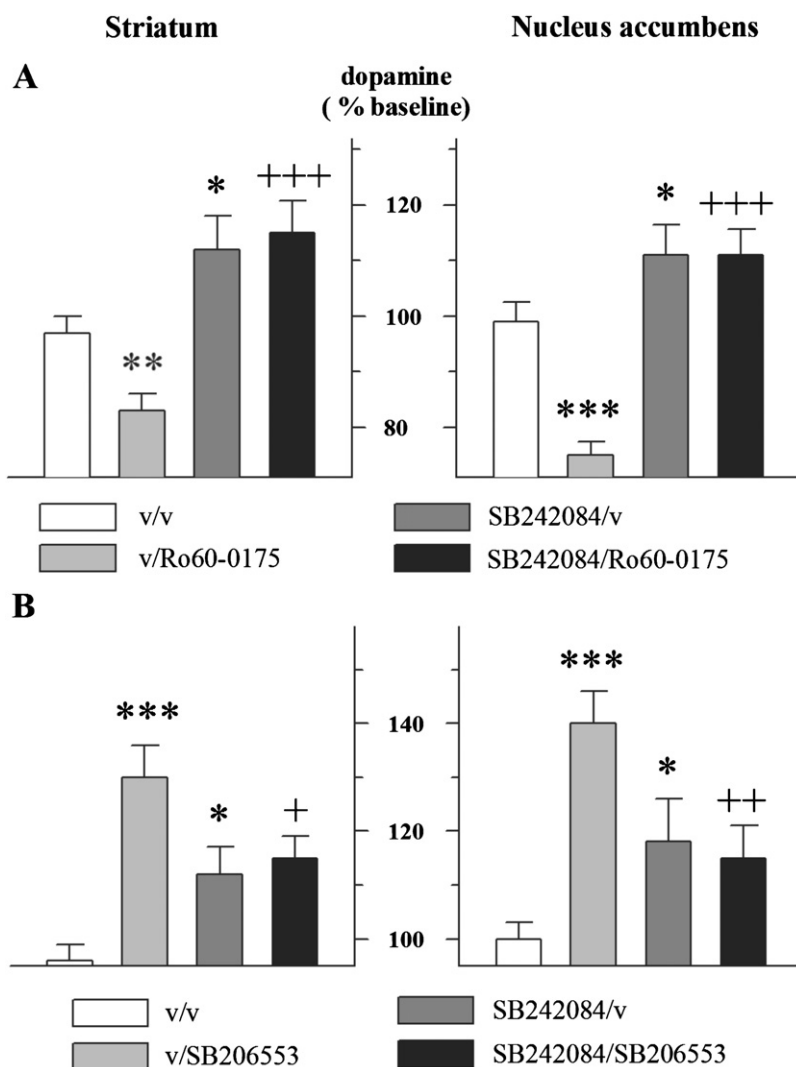


Fig. 5. Effect of central 5-HT_{2C} receptor blockade on 5-HT_{2C} receptor-mediated regulation of DA release in vivo. Reversal by SB 242084 (1 mg/kg, i.p.) of the effects elicited by the inverse agonist SB 206553 (5 mg/kg, i.p.) (A) and the agonist Ro 60-0175 (3 mg/kg, i.p.) (B) on DA release in the rat striatum and the NAc. SB 242084 was administered 30 min before SB 206553 or Ro 60-0175. Data represent mean \pm SEM percentages of baseline averaged over 2 h of monitoring ($n = 6-9$ animals/group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the vehicle/vehicle (v/v) group and + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ vs. the v/SB 206553 or v/Ro 60-0175 groups (Fisher's PLSD test). Data reproduced with permission from De Deurwaerdère et al. (2004).

release (Fig. 5). As discussed elsewhere (De Deurwaerdère et al., 2004), the relative efficacy difference observed reflects distinct intrinsic pharmacological properties of SB 206553 and SB 242084 compounds. Indeed, as shown by in vitro experiments in cells that express the 5-HT_{2C} receptor, SB 206553, in contrast to SB 242084,

behaves as a strong inverse agonist at the PLC pathway (De Deurwaerdère et al., 2004). Consistent with in vitro findings, in vivo experiments have shown that SB 242084 prevents the increase in striatal and accumbal DA release induced by SB 206553 and reverses the decrease in DA release produced by the 5-HT_{2C} agonist Ro 60-0175

in both brain regions (Fig. 5). Furthermore, SB 206553-stimulated DA release is insensitive to the decrease in 5-HT terminal activity induced by either intra-raphe injections of 5,7-DHT neurotoxin or peripheral administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (De Deurwaerdère et al., 2004). In agreement with the pharmacological characteristics of inverse agonist activity (Berg et al., 2005), these findings altogether indicate that the effect of SB 206553 on *in vivo* DA release is independent of the changes in extracellular levels of 5-HT and is likely related to its inverse agonist action at central 5-HT_{2C} receptors, thus strengthening the identification of constitutive activity of 5-HT_{2C} receptor as a physiological mechanism controlling the excitability of midbrain DA neuron *in vivo*.

Further support to this conclusion comes from latest studies (Navailles et al., 2006a) assessing the influence of 5-HT_{2C} receptor inverse agonists and antagonists on the release of DA induced by the antipsychotic drugs haloperidol and clozapine (a strong inverse agonist, Berg et al., 2006). Indeed, the increase in DA release induced by haloperidol in the rat striatum and NAc is potentiated by the 5-HT_{2C} receptor inverse agonist SB 206553 but unaltered by the 5-HT_{2C} receptor antagonists SB 242084 and SB 243213. Conversely, the effect of clozapine is unaffected by SB 206553 but blocked by SB 242084 and SB 243213. These findings indeed provide clear evidence that 5-HT_{2C} receptor inverse agonists can have effects different than those of 5-HT_{2C} receptor antagonists *in vivo*. Also, they indicate that the constitutive activity of 5-HT_{2C} receptor participates in the dopaminergic effects of the antipsychotic drugs clozapine and haloperidol, and that clozapine modulates subcortical DA release by acting as a 5-HT_{2C} inverse agonist *in vivo*.

Interestingly, it has also been shown that the control exerted by the constitutive activity of 5-HT_{2C} receptor on DA neuron activity occurs in a region-dependent manner in the rat brain. Indeed, intracranial microinjection studies examining the relative contribution of VTA and NAc 5-HT_{2C} receptors in the control of accumbal DA release have recently revealed that the NAc may represent a primary site of action for the

effects of the constitutive activity of 5-HT_{2C} receptor in the regulation of the mesoaccumbens DA pathway (Navailles et al., 2006b). Intra-VTA injections of the 5-HT_{2C} receptor antagonists SB 242084 and/or SB 243213 prevent the decrease in accumbal DA outflow induced by the 5-HT_{2C} receptor agonist Ro 60-0175 but do not affect the increase in DA outflow induced by the 5-HT_{2C} receptor inverse agonist SB 206553. Intra-NAc infusions of SB 242084, as in the case of its peripheral administration (De Deurwaerdère et al., 2004), block both Ro 60-0175- and SB 206553-induced changes of DA outflow (Fig. 6). Thus, whereas both VTA and NAc 5-HT_{2C} receptors participate in the inhibitory control exerted by 5-HT_{2C} receptor agonist on accumbal DA release, 5-HT_{2C} receptors in the NAc are primarily involved in the tonic inhibitory control exerted by the constitutive activity of central 5-HT_{2C} receptor. In accordance with this conclusion, intra-NAc, but not intra-VTA, administration of SB 206553 increases basal DA outflow in the NAc (Navailles et al., 2006b). The observed region-dependent effect of the inverse agonist SB 206553 could be related to different levels of 5-HT_{2C} receptor constitutive activity in the VTA and the NAc related to the pre-RNA editing of the 5-HT_{2C} receptor. Indeed, as discussed elsewhere (Navailles et al., 2006b), region-dependent RNA editing of 5-HT_{2C} receptor (Burns et al., 1997) may represent a mechanism generating populations with different levels of constitutive activity (Niswender et al., 1999).

The findings reported above on the whole, while providing new insights into the dominant role of the 5-HT_{2C} receptor in the regulatory neurochemistry of central DA neuronal function, underline the relevance of the constitutive activity in regulating physiological systems *in vivo* and the need for a better understanding of the therapeutic potential of inverse agonism for pathological conditions depending upon DA neuron dysfunction (Milligan et al., 1995; Berg et al., 2005). In this regard, it is noteworthy that 5-HT_{2C} constitutive receptor activity has been hypothesized to play a role in the pathophysiology of depressive states and the therapeutic effects of antidepressant drugs (Millan, 2005).

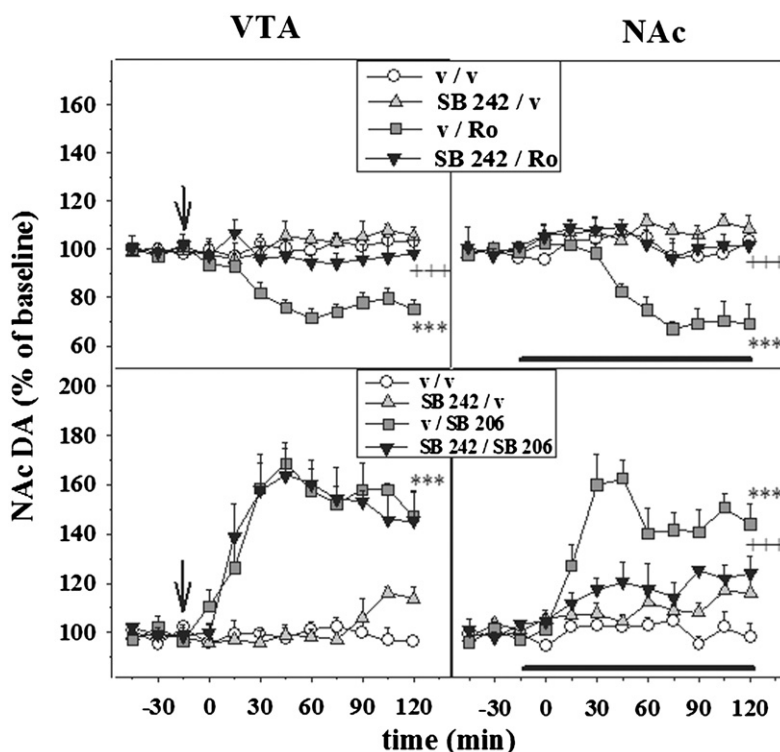


Fig. 6. Time course effect of the local administration in the ventral tegmental area (VTA, left panels) and nucleus accumbens (NAc, right panels) of the 5-HT_{2C} receptor antagonist SB 242084 (SB 242) on the changes of accumbal DA outflow induced by the peripheral administration of the 5-HT_{2C} receptor agonist Ro 60-0175 (Ro, upper panels) and the 5-HT_{2C} receptor inverse agonist SB 206553 (SB 206, lower panels). Ro and SB 206 were administered intraperitoneally at 3 and 5 mg/kg, respectively, at time zero. *Left panels*: SB 242 (0.5 µg/0.2 µl) was injected into the VTA (vertical arrows) 15 min before Ro or SB 206. *Right panels*: Intra-NAc perfusion of SB 242 (1 µM) by reverse dialysis started 15 min before Ro or SB 206 and was maintained until the end of the experiment (horizontal bars). Data are presented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration ($n = 4-8$ animals/group). *** $p < 0.001$ vs. the vehicle/vehicle (v/v) group and + + + $p < 0.001$ vs. the respective v/Ro or v/SB 206 group (Fisher's PLSD test). Data reproduced with permission by Blackwell Publishing, Oxon, from Navailles et al. (2006b).

Concluding remarks

It seems clear that both 5-HT_{2A} and 5-HT_{2C} receptors can display constitutive activity *in vivo*, which has physiological relevance for acquisition of an associative-learning response and for regulation of DA release, respectively. Furthermore, the discovery of drugs with inverse agonist properties, which reduce constitutive activity, allows for an additional dimension for control of 5-HT_{2A} and 5-HT_{2C} receptor activity and has greatly increased the richness of our pharmacological treasure chest. What is not clear, however, is the extent to which alterations in constitutive

activity of 5-HT_{2A} and 5-HT_{2C} receptors can contribute to pathologies such as depression. Until this issue is resolved, we cannot know whether the inverse agonist properties of some drugs may be exploited therapeutically. Further research is needed to determine if the properties of constitutive receptors and the actions of inverse agonists identified *in vitro* can be extended to *in vivo* systems. Some fruitful areas of continued research may include studies of prolonged treatment with inverse agonists, which have been shown to reduce constitutive desensitization and enhance signalling of heterologous receptor systems *in vitro*.

Abbreviations

AA	arachidonic acid
BOL	bromo-lysergic acid diethylamide
CR	conditioned response
DA	dopamine
5,7-DHT	5,7-dihydroxytryptamine
DOI	2,5-dimethoxy-4-iodophenylisopropylamine
DOM	2,5-dimethoxy-4-methylphenylisopropylamine
5-HT	5-hydroxytryptamine, serotonin
IP	inositol phosphates
LSD	lysergic acid diethylamide
MDA	3,4-methylenedioxymphetamine
MDMA	3,4-methylenedioxymethamphetamine
NAc	nucleus accumbens
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)-tertraline
PLA ₂	phospholipase A ₂
PLC	phospholipase C
SSRI	selective serotonin reuptake inhibitor
TFMPP	3-trifluoromethylphenylpiperazine
VTA	ventral tegmental area

Acknowledgements

The authors wish to acknowledge support from the National Institutes of Health (USPHS grants GM58652 and MH16841-40), the National Alliance for Research on Schizophrenia and Depression and the Institut National de la Santé et de la Recherche Médicale (INSERM)–Bordeaux 2 University.

References

- Aghajanian, G.K. and Marek, G.J. (2000) Serotonin model of schizophrenia: emerging role of glutamate mechanisms. *Brain Res. Brain Res. Rev.*, 31(2–3): 302–312.
- Aloyo, V.J., Dave, K.D., Rahman, T. and Harvey, J.A. (2001) Selective and divergent regulation of cortical 5-HT(2A) receptors in rabbit. *J. Pharmacol. Exp. Ther.*, 299(3): 1066–1072.

- Bagdy, G., Graf, M., Anheuer, Z.E., Modos, E.A. and Kantor, S. (2001) Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT_{2C} receptor antagonist SB-242084 but not the 5-HT_{1A} receptor antagonist WAY-100635. *Int. J. Neuropsychopharmacol.*, 4(4): 399–408.
- Bakish, D., Lapierre, Y.D., Weinstein, R., Klein, J., Wiens, A., Jones, B., Horn, E., Browne, M., Bourget, D., Blanchard, A., et al. (1993) Ritanserin, imipramine, and placebo in the treatment of dysthymic disorder. *J. Clin. Psychopharmacol.*, 13(6): 409–414.
- Barker, E.L., Westphal, R.S., Schmidt, D. and Sanders-Bush, E. (1994) Constitutively active 5-hydroxytryptamine_{2C} receptors reveal novel inverse agonist activity of receptor ligands. *J. Biol. Chem.*, 269(16): 11687–11690.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Benjamin, D., Lal, H. and Meyerson, L.R. (1990) The effects of 5-HT_{1B} characterizing agents in the mouse elevated plus-maze. *Life Sci.*, 47(3): 195–203.
- Benjamin, J., Geraci, M., McCann, U., Greenberg, B.D. and Murphy, D.L. (1999) Attenuated response to m-CPP and to pentagastrin after repeated m-CPP in panic disorder. *Psychopharmacology (Berl.)*, 143(2): 215–216.
- Berg, K.A. and Clarke, W.P. (2006) Development of functionally selective agonists as novel therapeutic agents. *Drug Discov. Today Ther. Strateg.*, 3(4): 421–428.
- Berg, K.A., Clarke, W.P., Sailstad, C., Saltzman, A. and Maayani, S. (1994) Signal transduction differences between 5-hydroxytryptamine type 2A and Type 2C receptor systems. *Mol. Pharmacol.*, 46(3): 477–485.
- Berg, K.A., Cropper, J.D., Niswender, C.M., Sanders-Bush, E., Emeson, R.B. and Clarke, W.P. (2001a) RNA-editing of the 5-HT_{2C} receptor alters agonist-receptor-effector coupling specificity. *Br. J. Pharmacol.*, 134(2): 386–392.
- Berg, K.A., Harvey, J.A., Spampinato, U. and Clarke, W.P. (2005) Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. *Trends Pharmacol. Sci.*, 26(12): 625–630.
- Berg, K.A., Maayani, S., Goldfarb, J., Scaramellini, C., Leff, P. and Clarke, W.P. (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.*, 54(1): 94–104.
- Berg, K.A., Navailles, S., Sanchez, T.A., Silva, Y.M., Wood, M.D., Spampinato, U. and Clarke, W.P. (2006) Differential effects of 5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxyl]-5-pyridyl]-carbamoyl]-6-trifluoro methylindone (SB 243213) on 5-hydroxytryptamine(2C) receptor-mediated responses. *J. Pharmacol. Exp. Ther.*, 319(1): 260–268.
- Berg, K.A., Stout, B.D., Cropper, J.D., Maayani, S. and Clarke, W.P. (1999) Novel actions of inverse agonists on 5-HT_{2C} receptor systems. *Mol. Pharmacol.*, 55(5): 863–872.
- Berg, K.A., Stout, B.D., Maayani, S. and Clarke, W.P. (2001b) Differences in rapid desensitization of 5-hydroxytryptamine_{2A}

- and 5-hydroxytryptamine_{2C} receptor-mediated phospholipase C activation. *J. Pharmacol. Exp. Ther.*, 299(2): 593–602.
- Bhansali, P., Dunning, J., Singer, S.E., David, L. and Schmauss, C. (2007) Early life stress alters adult serotonin _{2C} receptor pre-mRNA editing and expression of the alpha subunit of the heterotrimeric G-protein G_q. *J. Neurosci.*, 27(6): 1467–1473.
- Bilkei-Gorzo, A., Gyertyan, I. and Levay, G. (1998) mCPP-induced anxiety in the light-dark box in rats – a new method for screening anxiolytic activity. *Psychopharmacology (Berl.)*, 136(3): 291–298.
- Boulougouris, V., Glennon, J.C. and Robbins, T.W. (2008) Dissociable effects of selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists on serial spatial reversal learning in rats. *Neuropsychopharmacology*, 33: 2007–2019.
- Bourne, H.R. (1997) How receptors talk to trimeric G proteins. *Curr. Opin. Cell Biol.*, 9(2): 134–142.
- Bristow, L.J., O'Connor, D., Watts, R., Duxon, M.S. and Hutson, P.H. (2000) Evidence for accelerated desensitisation of 5-HT_{2C} receptors following combined treatment with fluoxetine and the 5-HT_{1A} receptor antagonist, WAY 100,635, in the rat. *Neuropharmacology*, 39(7): 1222–1236.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Buchanan, R.W., Freedman, R., Javitt, D.C., Abi-Dargham, A. and Lieberman, J.A. (2007) Recent advances in the development of novel pharmacological agents for the treatment of cognitive impairments in schizophrenia. *Schizophr. Bull.*, 33(5): 1120–1130.
- Burns, C.M., Chu, H., Rueter, S.M., Hutchinson, L.K., Canton, H., Sanders-Bush, E. and Emeson, R.B. (1997) Regulation of serotonin-_{2C} receptor G-protein coupling by RNA editing. *Nature*, 387(6630): 303–308.
- Campbell, B.M. and Merchant, K.M. (2003) Serotonin _{2C} receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. *Brain Res.*, 993(1–2): 1–9.
- Cerione, R.A., Codina, J., Benovic, J.L., Lefkowitz, R.J., Birnbaumer, L. and Caron, M.G. (1984) The mammalian beta 2-adrenergic receptor: reconstitution of functional interactions between pure receptor and pure stimulatory nucleotide binding protein of the adenylate cyclase system. *Biochemistry*, 23(20): 4519–4525.
- Charney, D.S., Woods, S.W., Goodman, W.K. and Heninger, G.R. (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl.)*, 92(1): 14–24.
- Chidiac, P. (2002) Considerations in the evaluation of inverse agonism and protean agonism at G protein-coupled receptors. *Meth. Enzymol.*, 343: 3–16.
- Christopoulos, A. and Kenakin, T. (2002) G protein-coupled receptor allosterism and complexing. *Pharmacol. Rev.*, 54(2): 323–374.
- Cornelio, A.M. and Nunes-de-Souza, R.L. (2007) Anxiogenic-like effects of mCPP microinfusions into the amygdala (but not dorsal or ventral hippocampus) in mice exposed to elevated plus-maze. *Behav. Brain Res.*, 178(1): 82–89.
- Costa, T. and Herz, A. (1989) Antagonists with negative intrinsic activity at delta opioid receptors coupled to GTP-binding proteins. *Proc. Natl. Acad. Sci. U.S.A.*, 86(19): 7321–7325.
- Cremers, T.I., Giorgetti, M., Bosker, F.J., Hogg, S., Arnt, J., Mork, A., Honig, G., Bogeso, K.P., Westerink, B.H., den Boer, H., Wikstrom, H.V. and Tecott, L.H. (2004) Inactivation of 5-HT_{2C} receptors potentiates consequences of serotonin reuptake blockade. *Neuropsychopharmacology*, 29(10): 1782–1789.
- Cryan, J.F. and Lucki, I. (2000) Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295(3): 1120–1126.
- Dave, K.D., Harvey, J.A. and Aloyo, V.J. (2007) The time-course for up- and down-regulation of the cortical 5-hydroxytryptamine (5-HT)_{2A} receptor density predicts 5-HT_{2A} receptor-mediated behavior in the rabbit. *J. Pharmacol. Exp. Ther.*, 323(1): 327–335.
- De Deurwaerdere, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24(13): 3235–3241.
- De Deurwaerdere, P. and Spampinato, U. (2001) The nigrostriatal dopamine system: a neglected target for 5-HT_{2C} receptors. *Trends Pharmacol. Sci.*, 22(10): 502–504.
- de Mello Cruz, A.P., Pinheiro, G., Alves, S.H., Ferreira, G., Mendes, M., Faria, L., Macedo, C.E., Motta, V. and Landeira-Fernandez, J. (2005) Behavioral effects of systemically administered MK-212 are prevented by ritanserin microinfusion into the basolateral amygdala of rats exposed to the elevated plus-maze. *Psychopharmacology (Berl.)*, 182(3): 345–354.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin-_{2C/2B} receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91(2): 587–597.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-_{2C} receptors. *Neuroscience*, 103(1): 111–116.
- Dunlop, B.W. and Nemeroff, C.B. (2007) The role of dopamine in the pathophysiology of depression. *Arch. Gen. Psychiatry*, 64(3): 327–337.
- Dunlop, J., Sabb, A.L., Mazandarani, H., Zhang, J., Kalgaonker, S., Shukhina, E., Sukoff, S., Vogel, R.L., Stack, G., Schechter, L., Harrison, B.L. and Rosenzweig-Lipson, S. (2005) WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1h]indole], a novel 5-hydroxytryptamine _{2C} receptor-selective agonist with anorectic activity. *J. Pharmacol. Exp. Ther.*, 313(2): 862–869.

- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384(2): 233–247.
- Egan, C., Herrick-Davis, K. and Teitler, M. (1998) Creation of a constitutively activated state of the 5-HT_{2A} receptor by site-directed mutagenesis: revelation of inverse agonist activity of antagonists. *Ann. N.Y. Acad. Sci.*, 861: 136–139.
- Englander, M.T., Dulawa, S.C., Bhansali, P. and Schmauss, C. (2005) How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. *J. Neurosci.*, 25(3): 648–651.
- Esposito, E. (2006) Serotonin-dopamine interaction as a focus of novel antidepressant drugs. *Curr. Drug Targets*, 7(2): 177–185.
- Fitzgerald, L.W., Iyer, G., Conklin, D.S., Krause, C.M., Marshall, A., Patterson, J.P., Tran, D.P., Jonak, G.J. and Hartig, P.R. (1999) Messenger RNA editing of the human serotonin 5-HT_{2C} receptor. *Neuropsychopharmacology*, 21(2 Suppl): 82S–90S.
- Fone, K.C., Shalders, K., Fox, Z.D., Arthur, R. and Marsden, C.A. (1996) Increased 5-HT_{2C} receptor responsiveness occurs on rearing rats in social isolation. *Psychopharmacology (Berl.)*, 123(4): 346–352.
- Friedman, A., Deri, I., Friedman, Y., Dremencov, E., Goutkin, S., Kravchinsky, E., Mintz, M., Levi, D., Overstreet, D.H. and Yadid, G. (2007) Decoding of dopaminergic mesolimbic activity and depressive behavior. *J. Mol. Neurosci.*, 32(1): 72–79.
- Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Lefkowitz, R.J. and Caron, M.G. (2004) Desensitization of G protein-coupled receptors and neuronal functions. *Annu. Rev. Neurosci.*, 27: 107–144.
- Ganguli, S.C., Park, C.G., Holtmann, M.H., Hadac, E.M., Kenakin, T.P. and Miller, L.J. (1998) Protean effects of a natural peptide agonist of the G protein-coupled secretin receptor demonstrated by receptor mutagenesis. *J. Pharmacol. Exp. Ther.*, 286(2): 593–598.
- Gatch, M.B. (2003) Discriminative stimulus effects of m-chlorophenylpiperazine as a model of the role of serotonin receptors in anxiety. *Life Sci.*, 73(11): 1347–1367.
- Giorgetti, M. and Tecott, L.H. (2004) Contributions of 5-HT_{2C} receptors to multiple actions of central serotonin systems. *Eur. J. Pharmacol.*, 488(1–3): 1–9.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36(3): 205–221.
- Gray, J.A. and Roth, B.L. (2007) Molecular targets for treating cognitive dysfunction in schizophrenia. *Schizophr. Bull.*, 33(5): 1100–1119.
- Griebel, G., Misslin, R., Pawlowski, M. and Vogel, E. (1991) m-Chlorophenylpiperazine enhances neophobic and anxious behaviour in mice. *Neuroreport*, 2(10): 627–629.
- Grotewiel, M.S. and Sanders-Bush, E. (1999) Differences in agonist-independent activity of 5-HT_{2A} and 5-HT_{2C} receptors revealed by heterologous expression. *Naunyn Schmiedeberg Arch. Pharmacol.*, 359(1): 21–27.
- Gudermann, T., Schoneberg, T. and Schultz, G. (1997) Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu. Rev. Neurosci.*, 20: 399–427.
- Gurevich, I., Tamir, H., Arango, V., Dwork, A.J., Mann, J.J. and Schmauss, C. (2002) Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. *Neuron*, 34(3): 349–356.
- Hackler, E.A., Turner, G.H., Gresch, P.J., Sengupta, S., Deutch, A.Y., Avison, M.J., Gore, J.C. and Sanders-Bush, E. (2007) 5-Hydroxytryptamine_{2C} receptor contribution to m-chlorophenylpiperazine and N-methyl-beta-carboline-3-carboxamide-induced anxiety-like behavior and limbic brain activation. *J. Pharmacol. Exp. Ther.*, 320(3): 1023–1029.
- Harvey, J.A. (2003) Role of the serotonin 5-HT_{2A} receptor in learning. *Learn. Mem.*, 10(5): 355–362.
- Harvey, J.A., Quinn, J.L., Liu, R., Aloyo, V.J. and Romano, A.G. (2004) Selective remodeling of rabbit frontal cortex: relationship between 5-HT_{2A} receptor density and associative learning. *Psychopharmacology (Berl.)*, 172(4): 435–442.
- Harvey, J.A., Welsh, S.E., Hood, H. and Romano, A.G. (1999) Effect of 5-HT₂ receptor antagonists on a cranial nerve reflex in the rabbit: evidence for inverse agonism. *Psychopharmacology (Berl.)*, 141(2): 162–168.
- Herrick-Davis, K., Grinde, E. and Niswender, C.M. (1999) Serotonin 5-HT_{2C} receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. *J. Neurochem.*, 73(4): 1711–1717.
- Herrick-Davis, K., Grinde, E. and Teitler, M. (2000) Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295(1): 226–232.
- Invernizzi, R.W., Pierucci, M., Calcagno, E., Di Giovanni, G., Di Matteo, V., Benigno, A. and Esposito, E. (2007) Selective activation of 5-HT_{2C} receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience*, 144(4): 1523–1535.
- Iwamoto, K. and Kato, T. (2003) RNA editing of serotonin 2C receptor in human postmortem brains of major mental disorders. *Neurosci. Lett.*, 346(3): 169–172.
- Iwamoto, K., Nakatani, N., Bundo, M., Yoshikawa, T. and Kato, T. (2005) Altered RNA editing of serotonin 2C receptor in a rat model of depression. *Neurosci. Res.*, 53(1): 69–76.
- Jenck, F., Moreau, J.L., Mutel, V. and Martin, J.R. (1994) Brain 5-HT_{1C} receptors and antidepressants. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 18(3): 563–574.
- Jones, N., Duxon, M.S. and King, S.M. (2002) 5-HT_{2C} receptor mediation of unconditioned escape behaviour in the unstable elevated exposed plus maze. *Psychopharmacology (Berl.)*, 164(2): 214–220.

- Kahn, R.S. and Wetzler, S. (1991) m-Chlorophenylpiperazine as a probe of serotonin function. *Biol. Psychiatry*, 30(11): 1139–1166.
- Kellar, K.J., Cascio, C.S., Butler, J.A. and Kurtzke, R.N. (1981) Differential effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain. *Eur. J. Pharmacol.*, 69(4): 515–518.
- Kenakin, T. (1995) Agonist-receptor efficacy. II. Agonist trafficking of receptor signals. *Trends Pharmacol. Sci.*, 16(7): 232–238.
- Kenakin, T. (2001) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. *FASEB J.*, 15(3): 598–611.
- Kenakin, T. (2002) Efficacy at G-protein-coupled receptors. *Nat. Rev. Drug Discov.*, 1(2): 103–110.
- Kennett, G.A., Bailey, F., Piper, D.C. and Blackburn, T.P. (1995) Effect of SB 200646A, a 5-HT_{2C}/5-HT_{2B} receptor antagonist, in two conflict models of anxiety. *Psychopharmacology (Berl.)*, 118(2): 178–182.
- Kennett, G.A., Whitton, P., Shah, K. and Curzon, G. (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT_{1C} receptor antagonists. *Eur. J. Pharmacol.*, 164(3): 445–454.
- Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, V., Avenell, K.Y., Stean, T., Upton, N., Bromidge, S., Forbes, I.T., Brown, A.M., Middlemiss, D.N. and Blackburn, T.P. (1997) SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacology*, 36(4–5): 609–620.
- Klein, E., Zohar, J., Geraci, M.F., Murphy, D.L. and Uhde, T.W. (1991) Anxiogenic effects of m-CPP in patients with panic disorder: comparison to caffeine's anxiogenic effects. *Biol. Psychiatry*, 30(10): 973–984.
- Lafaille, F., Welner, S.A. and Suranyi-Cadotte, B.E. (1991) Regulation of serotonin type 2 (5-HT₂) and beta-adrenergic receptors in rat cerebral cortex following novel and classical antidepressant treatment. *J. Psychiatry Neurosci.*, 16(4): 209–214.
- Lane, J.R., Powney, B., Wise, A., Rees, S. and Milligan, G. (2007) Protean agonism at the dopamine D₂ receptor: (S)-3-(3-hydroxyphenyl)-N-propylpiperidine is an agonist for activation of G_{o1} but an antagonist/inverse agonist for G_{i1}, G_{i2}, and G_{i3}. *Mol. Pharmacol.*, 71(5): 1349–1359.
- Lee, T.W., Cotecchia, S. and Milligan, G. (1997) Up-regulation of the levels of expression and function of a constitutively active mutant of the hamster alpha_{1B}-adrenoceptor by ligands that act as inverse agonists. *Biochem. J.*, 325(Pt 3): 733–739.
- Leff, P. (1995) The two-state model of receptor activation. *Trends Pharmacol. Sci.*, 16: 89–97.
- Leff, P., Scaramellini, C., Law, C. and McKechnie, K. (1997) A three-state model of agonist action. *Trends Pharmacol. Sci.*, 18(10): 355–362.
- Leysen, J.E. (2004) 5-HT₂ receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3(1): 11–26.
- Lowy, M.T. and Meltzer, H.Y. (1988) Stimulation of serum cortisol and prolactin secretion in humans by MK-212, a centrally active serotonin agonist. *Biol. Psychiatry*, 23(8): 818–828.
- MacEwan, D.J. and Milligan, G. (1996) Inverse agonist-induced up-regulation of the human beta₂-adrenoceptor in transfected neuroblastoma X glioma hybrid cells. *Mol. Pharmacol.*, 50(6): 1479–1486.
- Marek, G.J., Carpenter, L.L., McDougale, C.J. and Price, L.H. (2003) Synergistic action of 5-HT_{2A} antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology*, 28(2): 402–412.
- Marek, G.J., Martin-Ruiz, R., Abo, A. and Artigas, F. (2005) The selective 5-HT_{2A} receptor antagonist M100907 enhances antidepressant-like behavioral effects of the SSRI fluoxetine. *Neuropsychopharmacology*, 30(12): 2205–2215.
- Martin, J.R., Ballard, T.M. and Higgins, G.A. (2002) Influence of the 5-HT_{2C} receptor antagonist, SB-242084, in tests of anxiety. *Pharmacol. Biochem. Behav.*, 71(4): 615–625.
- Martin, J.R., Bos, M., Jenck, F., Moreau, J., Mutel, V., Sleight, A.J., Wichmann, J., Andrews, J.S., Berendsen, H.H., Broekkamp, C.L., Ruigt, G.S., Kohler, C. and Delft, A.M. (1998) 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.*, 286(2): 913–924.
- McGrew, L., Price, R.D., Hackler, E., Chang, M.S. and Sanders-Bush, E. (2004) RNA editing of the human serotonin 5-HT_{2C} receptor disrupts transactivation of the small G-protein RhoA. *Mol. Pharmacol.*, 65(1): 252–256.
- Mengod, G., Palacios, J.M., Wiederhold, K.H. and Hoyer, D. (1997) 5-hydroxytryptamine receptor histochemistry: comparison of receptor mRNA distribution and radioligand autoradiography in the brain. In: Baumgarten H.G. and Gothert M. (Eds.), *Serotonergic Neurons and 5-HT Receptors in the CNS*. Springer-Verlag, Berlin, pp. 213–237.
- Millan, M.J. (2005) Serotonin 5-HT_{2C} receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie*, 60(5): 441–460.
- Millan, M.J. (2006a) Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol. Ther.*, 110(2): 135–370.
- Millan, M.J. (2006b) Serotonin 5-HT_{2C} receptors as a target for the treatment of depressive and anxious states: Focus on novel therapeutic strategies. *Therapie*, 60(5): 441–460.
- Milligan, G. and Bond, R.A. (1997) Inverse agonism and the regulation of receptor number. *Trends Pharmacol. Sci.*, 18(12): 468–474.
- Milligan, G., Bond, R.A. and Lee, M. (1995) Inverse agonism: pharmacological curiosity or potential therapeutic strategy? *Trends Pharmacol. Sci.*, 16: 10–13.
- Mitchell, P.J. and Redfern, P.H. (2005) Animal models of depressive illness: the importance of chronic drug treatment. *Curr. Pharm. Des.*, 11(2): 171–203.
- Moreau, J.L., Bos, M., Jenck, F., Martin, J.R., Mortas, P. and Wichmann, J. (1996) 5HT_{2C} receptor agonists exhibit antidepressant-like properties in the anhedonia model of depression in rats. *Eur. Neuropsychopharmacol.*, 6(3): 169–175.

- Moreau, J.L., Jenck, F., Martin, J.R., Perrin, S. and Haefely, W.E. (1993) Effects of repeated mild stress and two antidepressant treatments on the behavioral response to 5HT_{1C} receptor activation in rats. *Psychopharmacology (Berl.)*, 110(1–2): 140–144.
- Moreau, X., Jeanningros, R. and Mazzola-Pomietto, P. (2001) Chronic effects of triiodothyronine in combination with imipramine on 5-HT transporter, 5-HT_{1A} and 5-HT_{2A} receptors in adult rat brain. *Neuropsychopharmacology*, 24(6): 652–662.
- Moya, P.R., Berg, K.A., Gutierrez-Hernandez, M.A., Saez-Briones, P., Reyes-Parada, M., Cassels, B.K. and Clarke, W.P. (2007) Functional selectivity of hallucinogenic phenethylamine and phenylisopropylamine derivatives at human 5-HT_{2A} and 5-HT_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 321(3): 1054–1061.
- Mueller, E.A., Murphy, D.L. and Sunderland, T. (1985) Neuroendocrine effects of M-chlorophenylpiperazine, a serotonin agonist, in humans. *J. Clin. Endocrinol. Metab.*, 61(6): 1179–1184.
- Navailles, S., Deurwaerdere, P. and Spampinato, U. (2006a) Clozapine and haloperidol differentially alter the constitutive activity of central serotonin_{2C} receptors in vivo. *Biol. Psychiatry*, 59(6): 568–575.
- Navailles, S., Moison, D., Ryczko, D. and Spampinato, U. (2006b) Region-dependent regulation of mesoaccumbens dopamine neurons in vivo by the constitutive activity of central serotonin_{2C} receptors. *J. Neurochem.*, 99(4): 1311–1319.
- Neubig, R.R. (2007) Missing links: mechanisms of protean agonism. *Mol. Pharmacol.*, 71(5): 1200–1202.
- Newman-Tancredi, A., Cussac, D., Marini, L., Touzard, M. and Millan, M.J. (2003) h5-HT_{1B} receptor-mediated constitutive Galphai3-protein activation in stably transfected Chinese hamster ovary cells: an antibody capture assay reveals protean efficacy of 5-HT. *Br. J. Pharmacol.*, 138(6): 1077–1084.
- Ni, Y.G. and Miledi, R. (1997) Blockage of 5HT_{2C} serotonin receptors by fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.*, 94(5): 2036–2040.
- Niswender, C.M., Copeland, S.C., Herrick-Davis, K., Emeson, R.B. and Sanders-Bush, E. (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, 274(14): 9472–9478.
- Niswender, C.M., Herrick-Davis, K., Dilley, G.E., Meltzer, H.Y., Overholser, J.C., Stockmeier, C.A., Emeson, R.B. and Sanders-Bush, E. (2001) RNA editing of the human serotonin 5-HT_{2C} receptor: alterations in suicide and implications for serotonergic pharmacotherapy. *Neuropsychopharmacology*, 24(5): 478–491.
- Palvimäki, E.P., Roth, B.L., Majasuo, H., Laakso, A., Kuoppamäki, M., Syvalähti, E. and Hietala, J. (1996) Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT_{2c} receptor. *Psychopharmacology (Berl.)*, 126(3): 234–240.
- Pauwels, P.J., Rauli, I., Wurch, T. and Colpaert, F.C. (2002) Evidence for protean agonism of RX 831003 at alpha 2A-adrenoceptors by co-expression with different G alpha protein subunits. *Neuropharmacology*, 42(6): 855–863.
- Perez, D.M. and Karnik, S.S. (2005) Multiple signaling states of G-protein-coupled receptors. *Pharmacol. Rev.*, 57(2): 147–161.
- Peroutka, S.J. and Snyder, S.H. (1980) Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. *Science*, 210(4465): 88–90.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E. and Spampinato, U. (2002) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 311–324.
- Price, R.D. and Sanders-Bush, E. (2000) RNA editing of the human serotonin 5-HT_{2C} receptor delays agonist-stimulated calcium release. *Mol. Pharmacol.*, 58(4): 859–862.
- Price, R.D., Weiner, D.M., Chang, M.S. and Sanders-Bush, E. (2001) RNA editing of the human serotonin 5-HT_{2C} receptor alters receptor-mediated activation of G13 protein. *J. Biol. Chem.*, 276(48): 44663–44668.
- Prisco, S., Pagannone, S. and Esposito, E. (1994) Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. *J. Pharmacol. Exp. Ther.*, 271(1): 83–90.
- Robinson, E.S., Dalley, J.W., Theobald, D.E., Glennon, J.C., Pezze, M.A., Murphy, E.R. and Robbins, T.W. (2008) Opposing roles for 5-HT_{2A} and 5-HT_{2C} receptors in the nucleus accumbens on inhibitory response control in the 5-choice serial reaction time task. *Neuropsychopharmacology*, advance online publication doi:10.1038/sj.npp.1301636.
- Romano, A.G., Hood, H. and Harvey, J.A. (2000) Dissociable effects of the 5-HT₂ antagonist mianserin on associative learning and performance in the rabbit. *Pharmacol. Biochem. Behav.*, 67(1): 103–110.
- Romano, A.G., Quinn, J.L., Liu, R., Dave, K.D., Schwab, D., Alexander, G., Aloyo, V.J. and Harvey, J.A. (2006) Effect of serotonin depletion on 5-HT_{2A}-mediated learning in the rabbit: evidence for constitutive activity of the 5-HT_{2A} receptor in vivo. *Psychopharmacology (Berl.)*, 184(2): 173–181.
- Rosenzweig-Lipson, S., Sabb, A., Stack, G., Mitchell, P., Lucki, I., Malberg, J.E., Grauer, S., Brennan, J., Cryan, J.F., Sukoff Rizzo, S.J., Dunlop, J., Barrett, J.E. and Marquis, K.L. (2007) Antidepressant-like effects of the novel, selective, 5-HT_{2C} receptor agonist WAY-163909 in rodents. *Psychopharmacology (Berl.)*, 192(2): 159–170.
- Roth, B.L., Hanizavareh, S.M. and Blum, A.E. (2004) Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology (Berl.)*, 174(1): 17–24.
- Roth, B.L., Nakaki, T., Chuang, D.M. and Costa, E. (1986) 5-Hydroxytryptamine₂ receptors coupled to phospholipase C in rat aorta: modulation of phosphoinositide turnover by phorbol ester. *J. Pharmacol. Exp. Ther.*, 238(2): 480–485.
- Roth, B.L., Willins, D.L., Kristiansen, K. and Kroeze, W.K. (1998) 5-Hydroxytryptamine₂-family receptors (5-hydroxytryptamine_{2A}, 5-hydroxytryptamine_{2B}, 5-hydroxytryptamine_{2C}): where structure meets function. *Pharmacol. Ther.*, 79(3): 231–257.

- Schmidt, C.J. and Fadayel, G.M. (1996) Regional effects of MK-801 on dopamine release: effects of competitive NMDA or 5-HT_{2A} receptor blockade. *J. Pharmacol. Exp. Ther.*, 277(3): 1541–1549.
- Schmidt, C.J., Fadayel, G.M., Sullivan, C.K. and Taylor, V.L. (1992) 5-HT₂ receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur. J. Pharmacol.*, 223(1): 65–74.
- Shapiro, D.A., Kristiansen, K., Weiner, D.M., Kroeze, W.K. and Roth, B.L. (2002) Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. *J. Biol. Chem.*, 277(13): 11441–11449.
- Simpson, L. and Emeson, R.B. (1996) RNA editing. *Annu. Rev. Neurosci.*, 19: 27–52.
- Smith, H.C., Gott, J.M. and Hanson, M.R. (1997) A guide to RNA editing. *RNA*, 3(10): 1105–1123.
- Southwick, S.M., Krystal, J.H., Bremner, J.D., Morgan, C.A., III, Nicolaou, A.L., Nagy, L.M., Johnson, D.R., Heninger, G.R. and Charney, D.S. (1997) Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch. Gen. Psychiatry*, 54(8): 749–758.
- Stevens, P.A., Bevan, N., Rees, S. and Milligan, G. (2000) Resolution of inverse agonist-induced up-regulation from constitutive activity of mutants of the α (1b)-adrenoceptor. *Mol. Pharmacol.*, 58(2): 438–448.
- Stockmeier, C.A. (2003) Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. *J. Psychiatr. Res.*, 37(5): 357–373.
- Stout, B.D., Clarke, W.P. and Berg, K.A. (2002) Rapid desensitization of the serotonin(2C) receptor system: effector pathway and agonist dependence. *J. Pharmacol. Exp. Ther.*, 302(3): 957–962.
- Teitler, M., Herrick-Davis, K. and Purohit, A. (2002) Constitutive activity of G-protein coupled receptors: emphasis on serotonin receptors. *Curr. Top. Med. Chem.*, 2(6): 529–538.
- Terry, A.V., Jr., Buccafusco, J.J. and Bartoszyk, G.D. (2005) Selective serotonin 5-HT_{2A} receptor antagonist EMD 281014 improves delayed matching performance in young and aged rhesus monkeys. *Psychopharmacology (Berl.)*, 179(4): 725–732.
- Urban, J.D., Clarke, W.P., von Zastrow, M., Nichols, D.E., Kobilka, B., Weinstein, H., Javitch, J.A., Roth, B.L., Christopoulos, A., Sexton, P.M., Miller, K.J., Spedding, M. and Mailman, R.B. (2007) Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharmacol. Exp. Ther.*, 320(1): 1–13.
- Van Oekelen, D., Luyten, W.H. and Leysen, J.E. (2003) 5-HT_{2A} and 5-HT_{2C} receptors and their atypical regulation properties. *Life Sci.*, 72(22): 2429–2449.
- Wang, Q., O'Brien, P.J., Chen, C.X., Cho, D.S., Murray, J.M. and Nishikura, K. (2000) Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin_{2C} receptors. *J. Neurochem.*, 74(3): 1290–1300.
- Weiner, D.M., Burstein, E.S., Nash, N., Croston, G.E., Currier, E.A., Vanover, K.E., Harvey, S.C., Donohue, E., Hansen, H.C., Andersson, C.M., Spalding, T.A., Gibson, D.F., Krebs-Thomson, K., Powell, S.B., Geyer, M.A., Hacksell, U. and Brann, M.R. (2001) 5-hydroxytryptamine_{2A} receptor inverse agonists as antipsychotics. *J. Pharmacol. Exp. Ther.*, 299(1): 268–276.
- Welsh, S.E., Kachelries, W.J., Romano, A.G., Simansky, K.J. and Harvey, J.A. (1998) Effects of LSD, ritanserin, 8-OH-DPAT, and lisuride on classical conditioning in the rabbit. *Pharmacol. Biochem. Behav.*, 59(2): 469–475.
- Wess, J. (1998) Molecular basis of receptor/G-protein-coupling selectivity. *Pharmacol. Ther.*, 80(3): 231–264.
- Westphal, R.S., Backstrom, J.R. and Sanders-Bush, E. (1995) Increased basal phosphorylation of the constitutively active serotonin 2C receptor accompanies agonist-mediated desensitization. *Mol. Pharmacol.*, 48(2): 200–205.
- Wilbanks, A.M., Laporte, S.A., Bohn, L.M., Barak, L.S. and Caron, M.G. (2002) Apparent loss-of-function mutant GPCRs revealed as constitutively desensitized receptors. *Biochemistry*, 41(40): 11981–11989.
- Williams, G.V., Rao, S.G. and Goldman-Rakic, P.S. (2002) The physiological role of 5-HT_{2A} receptors in working memory. *J. Neurosci.*, 22(7): 2843–2854.
- Willins, D.L., Berry, S.A., Alsayegh, L., Backstrom, J.R., Sanders-Bush, E., Friedman, L. and Roth, B.L. (1999) Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors in vitro and in vivo. *Neuroscience*, 91(2): 599–606.
- Wood, M.D. (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.*, 2(6): 383–387.
- Wood, M.D., Reavill, C., Trail, B., Wilson, A., Stean, T., Kennett, G.A., Lightowler, S., Blackburn, T.P., Thomas, D., Gager, T.L., Riley, G., Holland, V., Bromidge, S.M., Forbes, I.T. and Middlemiss, D.N. (2001) SB-243213; a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. *Neuropharmacology*, 41(2): 186–199.
- Yang, W., Wang, Q., Kanes, S.J., Murray, J.M. and Nishikura, K. (2004) Altered RNA editing of serotonin 5-HT_{2C} receptor induced by interferon: implications for depression associated with cytokine therapy. *Brain Res. Mol. Brain Res.*, 124(1): 70–78.

CHAPTER 15

The role of dopamine and serotonin in suicidal behaviour and aggression

Erik Ryding^{1,2,*}, Mats Lindström³ and Lil Träskman-Bendz³

¹Department of Clinical Neurophysiology, Karolinska Hospital, Huddinge, SE 141 52 Stockholm, Sweden

²Section of Clinical Neurophysiology, Department of Neuroscience, University Hospital of Lund, SE 221 85 Lund, Sweden

³Section of Psychiatry, Department of Clinical Neuroscience, University Hospital of Lund, SE 221 85 Lund, Sweden

Abstract: Serotonin and dopamine are two monoamines which are known to interact with each other. Their role for suicidal behaviour, aggression and mood are reviewed in this chapter. We found a substantial amount of evidence for the relevance of a serotonin and dopamine model of aggression, and for aggression as a major risk factor for suicide. Evidence was found that serotonin and dopamine also may be involved in depressed mood, and possibly the individual's ability to cope with imminent suicidality.

Keywords: dopamine; serotonin; suicide; aggression

Introduction

The concept of suicide is closely related to mood disorders. However, only about half of those who make serious suicide attempts are depressed (Roy, 1982; Isacson et al., 1994; Mann et al., 1999; Zonda, 2006). Apart from psychiatric diagnoses, such as unipolar depressive disorder, anxiety disorder, bipolar disease, schizophrenia, alcohol and/or substance abuse, borderline personality disorder, phenomena such as childhood abuse, grief, religious, political or socio-economic aspects, as well as physical disease like cancer, HIV and epilepsy are associated with suicidality. In order to predict and prevent suicidal behaviour, much effort has been spent to find a common denominator for those who make suicide attempts.

Suicidal ideation, often associated with depression, has been an obvious and well-studied parameter. Ideation about suicide is related to an increased risk for suicide attempts, especially when there has been previous attempt (Kuo et al., 2001; De Leo et al., 2002; Spirito et al., 2003). Suicidal ideation is, however, much more common than suicide attempts. Smith and Crawford (1986) found that 63% of a high school population reported suicidal ideation, while the actual frequency of suicide attempts among them was 8%. Consequently, suicidal ideation has a limited value as a risk factor for suicide in the general population (Busch et al., 2003).

Biochemical findings related to suicidal behaviour, depression and violence have mainly involved serotonin (5-hydroxytryptamine, 5-HT) and dopamine (DA) (Träskman et al., 1981; Apter et al., 1990; Engström et al., 1999; Träskman-Bendz and Mann, 2000; Mann, 2003; Pitchot et al., 2001a, b, c, 2003). Specifically, a coupling between 5-HT dysfunction and violent, impulsive,

*Corresponding author. Tel: +46858587382;
Fax: +46 08 585 820 30; E-mail: erik.ryding@karolinska.se

aggressive and suicidal behaviour has been demonstrated (Träskman et al., 1981; Coccaro et al., 1989; Mann et al., 1992), but there are also indications of a positive correlation to dopamine, as indicated by the metabolite homovanillic acid (HVA) in cerebrospinal fluid (Soderstrom et al., 2001). An early finding identified a correlation between low monoamine oxidase (MAO) activity in blood platelets and an increased risk for suicide both in depressed and non-depressed subjects (Buchsbaum et al., 1976; Verkes et al., 1998; Skondras et al., 2004). Later studies of the gene coding for MAO have limited the association between MAO and suicidal behaviour to the subtype MAO-A (Shih et al., 1999; Du et al., 2002). Maltreated children with high levels of MAO-A expression have been less likely to develop antisocial violent behaviour than those with low MAO-A expression levels (Caspi et al., 2002).

Genetic studies of polymorphisms of the serotonin promoter region and their relation to suicide frequency have provided conflicting evidence (Arango et al., 2003; Baca-Garcia et al., 2004; Correa et al., 2004; Zalsman et al., 2005, 2006; Helbecque et al., 2006), but concerning the serotonin transporter (5-HTT), a possible significant association was found with a higher frequency of violent suicide attempts in a sub-population which had the short transporter allele (Neves et al., 2008; Wasserman et al., 2007).

Aggression and suicidal behaviour

McCloskey et al. (2008) report that subjects with intermittent explosive disorder (IED) in a structured interview show a severely increased risk for self-aggression, with about 13% reported suicide attempts and 7% non-lethal destructive behaviour. While determining the risk of suicide attempt in psychiatric patients, Mann et al. (2008) found that although a 'recent' suicidal attempt was best predicted by a current suicidal ideation, remote' attempters were best identified by lifetime aggression and 'subjective depression'. Especially in young individuals, high levels of impulsive-aggressive traits play a greater role in suicide occurrence (O'Donnell et al., 2005; McGirr et al., 2007).

In studies of the mental condition related to suicidal behaviour of hospitalized suicide attempters, depression, feelings of anger, impulsivity and aggression, especially in combination with alcohol abuse, stand out (Mann et al., 1999). Although anger and impulsivity may also be part of the symptoms of borderline personality disorder (Keilp et al., 2006), aggression still remains as an important discriminating symptom for many subjects at risk for repeated suicide attempts (Mann et al., 1999; Tureki, 2005). Also in depressive disorder, aggression is a major risk factor for suicidal behaviour, especially in subjects with a history of alcoholism (Dumais et al., 2005; Sher et al., 2005; Keilp et al., 2006).

Serotonin and dopamine in aggression

Pre-clinical, animal research

The first reports concerning serotonin and aggression in animals stem from about 1960 to 1970. Several different models of aggression were tested, for example shock-induced fighting, reacting aggressively to intrusion from foreign individuals of their own species, isolation, food competition and septal lesion-induced hyperirritability, and all with involvement of monoamines (Bernard, 1975; Kostowski et al., 1975; Daruna and Kent, 1976; Malick and Barnett, 1976). It was noted at an early stage that serotonin appeared to decrease aggression in several different aggression models and, inversely, that serotonin deficiency augmented aggression (Kostowski et al., 1975; Daruna and Kent, 1976; Malick and Barnett, 1976; Valzelli, 1982). Later studies have given a more detailed view, indicating that stimulating 5-HT_{1A} or 5-HT_{1B} receptors in the prefrontal antero-medial or ventral orbitofrontal cortical regions, or the dorsal raphe nucleus (with projection of serotonergic nerve fibres to the prefrontal regions), reduces aggression in rats or mice reacting to intrusion from foreign individuals of their species (de Boer et al., 2000; de Almeida et al., 2006; Bannai et al., 2007). Further investigations with intra-cerebral microdialysis have led to the formulation of a serotonin-dopamine model of regulation of aggression.

The model is based on a dynamic interaction between the amygdala, nucleus accumbens, and prefrontal cortex. Accumbal dopamine release, with concurrent aggression, is triggered by amygdala activation, and suppressed from prefrontal antero-medial or ventral orbitofrontal cortical regions, activated by serotonin receptors (van Erp and Miczek, 2000; Jackson and Moghaddam, 2001; Ferrari et al., 2003). Alcohol intake increases aggression, with concomitant increase of accumbal dopamine release (Fish et al., 1999; Miczek and de Almeida, 2001; van Erp and Miczek, 2007).

Human brain

In the human brain, the orbitofrontal region appears also to be involved in regulation of aggression and affect, because lesions in this region may cause loss of impulse control and anger (Berlin et al., 2004). When brain imaging was used to study human brain function in vivo, further support for involvement of the prefrontal regions in aggression and suicide was found. An inverse correlation between impulsiveness/initiative and the 5-HTT binding potential in the right orbitofrontal region was found for suicide attempters by Ryding et al. (2006) using ^{123}I - β -CIT and single photon emission computed tomography (SPECT). Frankle et al. (2005), using positron emission tomography (PET) and [^{11}C]McN5652 with affinity for the 5-HTT, found reduced 5-HTT in the anterior cingulate cortex in impulsive-aggressive subjects. Using a 5-HT_{2A} receptor ligand and SPECT, Audenaert et al. (2001) and van Heeringen et al. (2003) found a decreased binding index (indicating a decreased number of free receptors) in the frontal regions of violent suicide attempters. Audenaert et al. (2002) also found decreased frontal activation, as measured by cerebral blood flow, in depressed suicide attempters.

Genetic variations of the serotonin 1B receptor, which in animal models are related to reduced impulse/aggression control, were studied in human suicide victims and found to be significantly associated with a history of impulsive-aggressive behaviour (Zouk et al., 2007).

The fact that dopamine relates to increased human aggression is well known, i.e. from subjects abusing amphetamine and/or cocaine, both of which increase the brain synaptic dopamine concentration in nucleus accumbens (Telang et al., 1999; Goldstein et al., 2005; Boileau et al., 2007). Also, alcoholism is linked to aggression and dopamine (Tupala and Tiihonen, 2004; Gerevich et al., 2007; Tremblay et al., 2007; Denson et al., 2008).

High cerebro spinal fluid (CSF) levels of the dopamine metabolite HVA correlates with human aggression (Soderstrom et al., 2001). The relation between dopamine and inward directed aggression in non-depressed patients, expressed as suicide attempts, was studied by comparisons with non-attempters for growth hormone response to apomorphine (a dopaminergic agonist), which was found to be significantly lower in suicide attempters (Pitchot et al., 2001b).

Dopamine and serotonin in suicidal behaviour and depressive disorder

Post-mortem findings in the brain of suicide victims have been conflicting. A decreased 5-HTT and/or 5-HT_{1A} and 5-HT_{2A} serotonin receptor binding was reported by Stanley and Mann (1983), Gross-Isserdorff et al. (1989), Leake et al. (1991), Arango et al. (1997) and Mann et al. (2000), while post-mortem studies by Owen et al. (1986), Arora and Meltzer (1989), Lawrence et al. (1990), Hrdina et al. (1993) and Lowther et al. (1997) found no change in 5-HTT. Arató et al. (1987) found a laterality of frontal serotonergic neurons, which differs between suicide victims and humans who died of natural causes. A higher number of free [^3H]-imipramine binding sites (found on serotonergic neurons) were recorded in the left frontal cortex of suicide victims than in control subjects. This has later been replicated by Demeter et al. (1989) and Arató et al. (1991).

Also, in vivo studies give conflicting evidence concerning the role of dopamine and serotonin in serious suicide attempters. Leyton et al. (2006), in a PET study, found reduced orbital and ventral medial prefrontal alpha-[^{11}C]methyl-l-tryptophan trapping in patients who had made high-lethality

suicide attempts, indicating lower serotonin synthesis. [Ryding et al. \(2006\)](#) found no significant difference between serious suicide attempters and control subjects in regional serotonin or dopamine transporter levels, indicating no difference in synaptic concentrations. [Zalsman et al. \(2005\)](#) found no difference between suicidal and non-suicidal psychiatric in-patients in platelet 5-HTT binding.

A different aspect of the relation between suicide and serotonin/dopamine was indicated by [Zalsman et al. \(2005\)](#) who found a significant correlation between platelet 5-HTT binding and anger scores for suicide attempters, but not for control subjects. Similarly, [Ryding et al. \(2006\)](#) using SPECT and ^{123}I - β -CIT, found significant correlations between 5-HTT and test scores for impulsivity/initiative, and between the dopamine transporter (DAT) and scores for mental energy, for suicide attempters only, and not for control subjects. [Bah et al. \(2008\)](#) found significant correlations between the 5-HTT values found by [Ryding et al. \(2006\)](#) and polymorphisms in the gene coding for the 5-HTT, only in suicide attempters and not in control subjects.

Brain imaging studies of the effects of depression on dopamine and/or serotonin have reported conflicting findings.

Using ^{123}I - β -CIT and SPECT, [Willeit et al. \(2000\)](#) found reduced 5-HTT capacity in the hypothalamic and the thalamic regions of the brains of depressed drug-naïve adults. In a similar population, [Parsey et al. \(2006\)](#), using PET and a tracer substance with affinity for 5-HTT, found decreased 5-HTT capacity in the amygdala and midbrain regions. In contrast, [Dahlstrom et al. \(2000\)](#), using SPECT and ^{123}I - β -CIT, found an increased 5-HTT capacity in the hypothalamic/midbrain region for depressed drug-naïve children. Similarly, [Caspi et al. \(2003\)](#) found that individual homozygous with the long 5-HTT allele had less risk for depression caused by adverse life events than those homozygous or heterozygous with the short 5-HTT allele.

[Meyer et al. \(2004\)](#) found no difference in 5-HTT between patients with major depression and controls, using PET and [^{11}C]DSAB, except

for a subgroup with negativistic and dysfunctional attitudes.

Imaging studies with PET and SPECT of dopamine and depression indicate decreased striatal and extrastriatal synaptic dopamine levels, combined with increased free dopamine transporter levels ([Laasonen-Balk et al., 1999](#); [Brunswick et al., 2003](#); [Meyer et al., 2006](#); [Montgomery et al., 2007](#)).

It is of special interest that motor items in the Montgomery and Åsberg depression rating scale (MADRS; [Montgomery and Åsberg, 1979](#)) may be associated with striatal dopaminergic activity ([Koerts et al., 2007](#)). Consequently, varying levels of motor inhibition within or between depressed individuals may reflect the synaptic level of dopamine in the basal ganglia (and in nucleus accumbens?).

Concerning the relation between serotonin and dopamine, [Agren et al. \(1986\)](#) found evidence for a 'control' of serotonin turnover on dopamine turnover in cerebrospinal fluid metabolites 5-HIAA and HVA in patients with depressive disorders. [Tiihonen et al. \(1996\)](#) in a PET study of normal subjects, using the D2 receptor ligand [^{11}C]raclopride, found evidence for modulation of the synaptic striatal dopamine concentrations by the drug citalopram, which increases synaptic serotonin concentrations. With SPECT and ^{123}I - β -CIT, [Lindström et al. \(2004\)](#) found a positive correlation between whole brain 5-HTT and DAT capacity for suicide attempters, but not for control subjects. Using continuous cerebrospinal fluid sampling, [Geraciotti et al. \(1997\)](#) found a negative coupling between concentrations of the serotonin metabolite 5-HIAA and noradrenaline in normal subjects, which was lost in depressed patients. [Oreland et al. \(1981\)](#) found that there were significant correlations in healthy subjects between CSF concentrations of the dopamine metabolite HVA, the serotonin metabolite 5-HIAA, and MAO. This was not found in the depressed and suicidal patients. The interaction between serotonin, dopamine and noradrenaline during depressive episodes has consequently been a target for understanding the action mechanism of antidepressant drugs ([Bonhomme and Esposito, 1998](#); [Esposito, 2006](#)).

Conclusions

A substantial and growing amount of evidence indicates that the biological model of social aggression, based on findings in animals, may be relevant also for humans. Accordingly, serotonin decreases aggression through stimulation of prefrontal cortical regions that send inhibitory projections to the nucleus accumbens, which in aggression has been activated from the amygdala. In contrast, dopamine augments activation of the nucleus accumbens in aggression.

Increase in selective serotonin reuptake inhibitor (SSRI) medications, which increase the synaptic serotonin concentrations in the brain, has occurred within approximately the same time frame as a decrease in suicide rates, and a causative relationship has therefore been assumed, but is now debated (Isacsson et al., 1994; Gibbons et al., 2005, 2006). SSRI may even increase suicidal ideation (Hall and Lucke, 2006), but there is neither any conclusive evidence that SSRI increases suicide rates in adolescents nor any evidence of increases in violent suicides (Gibbons et al., 2006; Fazel et al., 2007).

Reduction of aggression may be a mechanism by which SSRI medication can decrease the suicide rate. Conversely, enhancement of brain dopamine concentrations, with use of drugs like alcohol, amphetamine or cocaine, may, through increased aggression, augment the suicide risk. Another aspect of dopamine-triggered aggression may be the well-known increased suicide risk when a depressed person improves along with reduction of motor and cognitive inhibition (Simon and Savarino, 2007). Because retarded depression may be due to low basal ganglia dopamine levels (Koerts et al., 2007), a disappearance of the inhibition may relate to a general brain dopamine increase, including within the accumbens nuclei, leading to increased aggression and suicide.

As was indicated by Mann et al. (1999), suicide risk appears to depend both on the pressures on the individual and the individual's ability to sustain. There have been few indications of biological factors, which result in a decreased ability to cope with imminent suicidality. Possibly the findings by Zalsman et al. (2005), Ryding et al.

(2006) and Bah et al. (2008) are relevant. They all report a difference between suicide attempters and control subjects, consisting of significant correlations, only in suicide attempters, between monoamine (mainly serotonin) transporter binding capacity (found in platelet or brain tissue) or type of gene coding for the 5-HTT and temperament test results. A possible explanation for this difference between suicide attempters and control subjects may be that suicide attempters lack normal intra-individual variations in serotonin and dopamine levels in response to surrounding demands (Ryding et al., 2006). A loss of, or decreased, serotonin and dopamine adaptability may give a decreased ability to cope with threatening situations, including imminent suicidality.

Abbreviations

CSF	cerebrospinal fluid
DA	dopamine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
5-HTT	serotonin transporter
HVA	homovanilic acid
IED	intermittent explosive disorder
MADRS	Montgomery Åsberg depression rating scale
MAO	monoamine oxidase
PET	positron emission tomography
SPECT	single photon emission computed tomography

Acknowledgements

The project was financially supported by the Swedish Research Council, no.: K2003-21KX-14548-01A and K2006-21X-14548-04-3, and the Segerfalk, E & H Sjöbring and OM Persson foundations.

References

- Agren, M., Mefford, I.N., Rudorfer, M.V., Linnoila, M. and Potter, W.Z. (1986) Interacting neurotransmitter systems. A non-experimental approach to the 5HAA-HVA correlation in human CSF. *J. Psychiatr. Res.*, 20(3): 175–193.

- Apter, A., van Praag, H.M., Plutchik, R., Sevy, S., Korn, M. and Brown, S.L. (1990) Interrelationships among anxiety, aggression, impulsivity, and mood: a serotonergically linked cluster? *Psychiatry Res.*, 32(2): 191–199.
- Arango, V., Huang, Y.Y., Underwood, M.D. and Mann, J.J. (2003) Genetics of the serotonergic system in suicidal behavior. *J. Psychiatr. Res.*, 37(5): 375–386.
- Arango, V., Underwood, M.D. and Mann, J.J. (1997) Postmortem findings in suicide victims. Implications for in vivo imaging studies. *Ann. N. Y. Acad. Sci.*, 836: 269–287.
- Arató, M., Frecska, E., MacCrimmon, D.J., Guscott, R., Saxena, B., Tekes, K. and Tothfalusi, L. (1991) Serotonergic interhemispheric asymmetry: neurochemical and pharmacologic evidence. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 15(6): 759–764.
- Arató, M., Tekes, K., Tothfalusi, L., Magyar, K., Palkovits, M., Demeter, E. and Falus, A. (1987) Serotonergic split brain and suicide. *Psychiatry Res.*, 21(4): 355–356.
- Arora, R.C. and Meltzer, H.Y. (1989) Serotonergic measures in the brains of suicide victims: 5-HT₂ binding sites in the frontal cortex of suicide victims and control subjects. *Am. J. Psychiatry*, 146(6): 730–736.
- Audenaert, K., Goethals, I., van Laere, K., Lahorte, P., Brans, B., Versijpt, J., Vervaeke, M., Beelaert, L., van Heeringen, C. and Dierckx, R. (2002) SPECT neuropsychological activation procedure with the Verbal Fluency Testing in attempted suicide patients. *Nucl. Med. Commun.*, 23(9): 907–916.
- Audenaert, K., van Laere, K., Dumont, F., Slegers, G., Mertens, J., van Heeringen, C. and Dierckx, R. (2001) Decreased frontal serotonin 5-HT_{2A} receptor binding index in deliberate self-harm patients. *Eur. J. Nucl. Med.*, 28(2): 175–182.
- Baca-Garcia, E., Vaquero, C., Diaz-Sastre, C., Garcia-Resa, E., Saiz-Ruiz, J., Fernandez-Piqueras, J. and de Leon, J. (2004) Lack of association between the serotonin transporter promoter gene polymorphism and impulsivity or aggressive behavior among suicide attempters and healthy volunteers. *Psychiatry Res.*, 126(2): 99–106.
- Bah, J., Lindström, M., Westberg, L., Mannerås, L., Ryding, E., Henningsson, S., Melke, J., Rosén, I., Träskman-Bendz, L. and Eriksson, E. (2008) Serotonin transporter gene polymorphisms: effect on serotonin transporter availability in the brain of suicide attempters. *Psychiatry Res.*, 162(3): 221–229.
- Bannai, M., Fish, E.W., Faccidomo, S. and Miczek, K.A. (2007) Anti-aggressive effects of agonists at 5-HT_{1B} receptors in the dorsal raphe nucleus of mice. *Psychopharmacology (Berl.)*, 193(2): 295–304.
- Berlin, H., Rolls, E. and Kischka, U. (2004) Impulsivity, time perception, emotion and reinforcement sensitivity in patients with orbitofrontal cortex lesions. *Brain*, 127(5): 1108–1126.
- Bernard, B.K. (1975) Aggression and the brain monoamines: what are the answers, but of more importance what are the questions ...? *Natl. Inst. Drug Abuse Res. Monogr. Ser.*, 12(3): 71–84.
- Boileau, I., Dagher, A., Leyton, M., Welfeld, K., Booij, L., Diksic, M. and Benkelfat, C. (2007) Conditioned dopamine release in humans: a positron emission tomography [¹¹C]raclopride study with amphetamine. *J. Neurosci.*, 27(15): 3998–4003.
- Bonhomme, N. and Esposito, E. (1998) Involvement of serotonin and dopamine in the mechanism of action of novel antidepressant drugs: a review. *J. Clin. Psychopharmacol.*, 18(6): 447–454.
- Brunswick, D.J., Amsterdam, J.D., Mozley, P.D. and Newberg, A. (2003) Greater availability of brain dopamine transporters in major depression shown by [99mTc]TRODAT-1 SPECT imaging. *Am. J. Psychiatry*, 160(10): 1836–1841.
- Buchsbaum, M.S., Coursey, R.D. and Murphy, D.L. (1976) The biochemical high-risk paradigm: behavioral and familial correlates of low platelet monoamine oxidase activity. *Science*, 194(4262): 339–341.
- Busch, K.A., Fawcett, J. and Jacobs, D.G. (2003) Clinical correlates of inpatient suicide. *J. Clin. Psychiatry*, 64(1): 14–19.
- Caspi, A., McClay, J., Moffitt, T.E., Mill, J., Martin, J., Craig, I.W., Taylor, A. and Poulton, R. (2002) Role of genotype in the cycle of violence in maltreated children. *Science*, 297(5582): 851–854.
- Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. and Poulton, R. (2003) Influence of the life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, 301(5631): 386–389.
- Coccaro, E.F., Siever, L.J., Klar, H.M., Maurer, G., Cochrane, K., Cooper, T.B., Mohs, R.C. and Davis, K.L. (1989) Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. *Arch. Gen. Psychiatry*, 47(7): 587–599.
- Correa, H., Campi-Azevedo, A.C., De Marco, L., Boston, W., Viana, M.M., Guimaraes, M.M., Costa, E., Miranda, D.M. and Romano-Silva, M.A. (2004) Familial suicide behavior: association with probands suicide attempt characteristics and 5-HTTLPR polymorphism. *Acta Psychiatr. Scand.*, 110(6): 459–464.
- Dahlstrom, M., Ahonen, A., Ebeling, H., Torniainen, P., Heikkilä, J. and Moilanen, I. (2000) Elevated hypothalamic/midbrain serotonin (monoamine) transporter availability in depressive drug-naïve children and adolescents. *Mol. Psychiatry*, 5(5): 514–522.
- Daruna, J.H. and Kent, E.W. (1976) Comparison of regional serotonin levels and turnover in the brain of naturally high and low aggressive rats. *Brain Res.*, 101(3): 489–501.
- de Almeida, R.M., Rosa, M.M., Santos, D.M., Saft, D.M., Benini, Q. and Miczek, K.A. (2006) 5-HT_{1B} receptors, ventral orbitofrontal cortex, and aggressive behaviors in mice. *Psychopharmacology (Berl.)*, 185(4): 441–450.
- de Boer, S.F., Lesourd, M., Mocaër, E. and Koolhaas, J.M. (2000) Somatodendritic 5-HT_{1A} autoreceptors mediate the anti-aggressive actions of 5-HT_{1A} receptor agonists in rats: an ethopharmacological study with S-15535, alnespirone, and WAY-1000635. *Neuropsychopharmacology*, 23(1): 20–23.

- de Leo, D., Padoani, W., Lonnqvist, J., Kerkhof, A.J., Bille-Brahe, U., Michel, K., Salander-Renberg, E., Schmidtke, A., Wasserman, D., Caon, F. and Scocco, P. (2002) Repetition of suicidal behaviour in elderly Europeans: a prospective longitudinal study. *J. Affect. Disord.*, 72(3): 291–295.
- Demeter, E., Tekes, K., Majorossy, K., Palkovits, M., Soos, M., Magyar, K. and Somogyi, E. (1989) The asymmetry of 3H-imipramine binding may predict psychiatric illness. *Life Sci.*, 44(19): 1403–1410.
- Denson, T.F., Aviles, F.E., Pollock, V.E., Earleywine, M., Vasquez, E.A. and Miller, N. (2008) The effects of alcohol and the salience of aggressive cues on triggered displaced aggression. *Aggress. Behav.*, 34(1): 25–33.
- Du, L., Faludi, G., Palkovits, M., Sotonyi, P., Bakish, D. and Hrdina, P.D. (2002) High activity-related allele of MAO-A gene associated with depressed suicide in males. *Neuroreport*, 13(9): 1195–1198.
- Dumais, A., Lesage, A.D., Alda, M., Rouleau, G., Dumont, M., Chawky, N., Roy, M., Mann, J.J., Benkelfat, C. and Tureki, G. (2005) Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. *Am. J. Psychiatry*, 162(11): 2116–2124.
- Engström, G., Alling, C., Blennow, K., Regnéll, G. and Träskman-Bendz, L. (1999) Reduced cerebrospinal HVA concentrations and HVA/5-HIAA ratios in suicide attempters. *Monoamine metabolites in 120 suicide attempters and 47 controls. Neuropsychopharmacology*, 9(5): 399–405.
- Esposito, E. (2006) Serotonin-dopamine interaction as focus of novel antidepressant drugs. *Curr. Drug Targets*, 7(2): 177–185.
- Fazel, S., Grann, M., Ahlner, J. and Goodwin, G. (2007) Suicides with violent means in individuals taking SSRIs and other antidepressants: a postmortem study in Sweden, 1992–2004. *J. Clin. Psychopharmacol.*, 27(5): 503–506.
- Ferrari, P.F., van Erp, A.M., Tornatzky, W. and Miczek, K.A. (2003) Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *Eur. J. Neurosci.*, 17(2): 371–378.
- Fish, E.W., Faccidomo, S. and Miczek, K.A. (1999) Aggression heightened by alcohol or social instigation in mice: reduction by the 5-HT(1B) receptor agonist CP-94,253. *Psychopharmacology (Berl.)*, 146(4): 391–399.
- Frankle, W.G., Lombardo, I., New, A.S., Goodman, M., Talbot, P.S., Huang, Y., Hwang, D.R., Slifstein, M., Curry, S., Abi-Dargham, A., Laruelle, M. and Siever, L.J. (2005) Brain serotonin transporter distribution in subjects with impulsive aggressivity: a positron emission study with [¹¹C]McN 5652. *Am. J. Psychiatry*, 162(5): 915–923.
- Geraciotti, T.D., Loosen, P.T., Ekhtator, N.N., Schmidt, D., Chambliss, B., Baker, D.G., Kasckow, J.W., Richtand, N.M., Keck, P.E. and Ebert, M.H. (1997) Uncoupling of serotonergic and noradrenergic systems in depression: preliminary evidence from continuous cerebrospinal fluid sampling. *Depress. Anxiety*, 6(3): 89–94.
- Gerevich, J., Bácskai, E. and Csobor, P. (2007) Aggression levels in treatment seeking inpatients with alcohol related problems compared to levels in the general population in Hungary. *J. Nerv. Ment. Dis.*, 195(8): 669–672.
- Gibbons, R.D., Hur, K., Bhaumik, D.K. and Mann, J.J. (2005) The relationship between antidepressant medication use and the rate of suicide. *Arch. Gen. Psychiatry*, 62(2): 165–172.
- Gibbons, R.D., Hur, K., Bhaumik, D.K. and Mann, J.J. (2006) The relationship between antidepressant prescription rates and rate of early adolescent suicide. *Am. J. Psychiatry*, 163(11): 1898–1904.
- Goldstein, R.Z., Alia-Klein, N., Leskovan, A.C., Fowler, J.S., Wang, G.J., Gur, R.C., Hitzemann, R. and Volkow, N.D. (2005) Anger and depression in cocaine addiction: association with the orbitofrontal cortex. *Psychiatry Res.*, 138(1): 13–22.
- Gross-Isserdorff, R., Israeli, M. and Biegon, A. (1989) Autoradiographic analysis of tritiated imipramine binding in the human brain post mortem: effects of suicide. *Arch. Gen. Psychiatry*, 46(3): 237–241.
- Hall, W.D. and Lucke, J. (2006) How have the selective serotonin reuptake inhibitor antidepressants affected suicide mortality? *Aust. N. Z. J. Psychiatry*, 40(11–12): 941–950.
- Helbecque, N., Sparks, D.L., Hunsaker, J.C., Jr. and Amouyel, P. (2006) The serotonin transporter promoter polymorphism and suicide. *Neurosci. Lett.*, 400(1–2): 13–15.
- Hrdina, P.D., Demeter, E., Vu, T.B., Sotonyi, P. and Palkovits, M. (1993) 5-HT uptake sites and 5-HT₂ receptors in brain of antidepressant-free suicide victims/depressives: increase in 5-HT₂ sites in cortex and amygdala. *Brain Res.*, 614(1–2): 37–44.
- Isacson, G., Holmgren, P., Wasserman, D. and Bergman, U. (1994) Use of antidepressants among people committing suicide in Sweden. *BMJ*, 308(6927): 506–509.
- Jackson, M.E. and Moghaddam, B. (2001) Amygdala regulation of nucleus accumbens dopamine output is governed by the prefrontal cortex. *J. Neurosci.*, 21(2): 676–681.
- Keilp, J.G., Goryn, M., Oquendo, M.A., Brodsky, B., Ellis, S.P., Stanley, B. and John Mann, J. (2006) Aggressiveness, not impulsiveness or hostility, distinguishes suicide attempters with major depression. *Psychol. Med.*, 36(12): 1779–1788.
- Koerts, J., Lendeers, K.L., Konig, M., Portman, A.T. and van Beilen, M. (2007) Striatal dopaminergic activity (FDOPA-PET) associated with cognitive items of a depression scale (MADRS) in Parkinson's disease. *Eur. J. Neurosci.*, 25(10): 3132–3136.
- Kostowski, W., Cxlonkowski, A., Markowdka, L. and Markiewicz, L. (1975) Intraspecific aggressiveness after lesions of midbrain raphe nuclei in rats. *Pharmacology*, 13(1): 81–85.
- Kuo, W.H., Gallo, J.J. and Tien, A.Y. (2001) Incidence of suicide ideation and attempts in adults: the 13-year follow-up of a community sample in Baltimore, Maryland. *Psychol. Med.*, 31(7): 1181–1191.
- Laasonen-Balk, T., Kuikka, J., Viinamäki, H., Husso-Saastamion, M., Lehtonen, J. and Tihiönen, J. (1999) Striatal dopamine transporter density in major depression. *Psychopharmacology (Berl.)*, 144(3): 282–285.
- Lawrence, K.M., de Paermentier, F., Cheetham, S.C., Crompton, M.R., Katona, C.L. and Horton, R.W. (1990) Brain 5-HT

- uptake sites, labelled with [3H]paroxetine, in antidepressant-free depressed suicides. *Brain Res.*, 526(1): 17–22.
- Leake, A., Fairbairn, A.F., McKeith, I.G. and Ferrier, I.N. (1991) Studies on the serotonin uptake binding site in major depressive disorder and control post-mortem brain: neurochemical and clinical correlates. *Psychiatry Res.*, 39(2): 155–165.
- Leyton, M., Parquette, V., Gravel, P., Rosa-Neto, P., Weston, F., Diksic, M. and Benkelfat, C. (2006) alpha-[¹¹C]Methyl-L-tryptophan trapping in orbital and ventral medial prefrontal cortex of suicide attempters. *Eur. Neuropsychopharmacol.*, 16(3): 220–223.
- Lindström, M.B., Ryding, E., Bosson, P., Ahnide, J.A., Rosén, I. and Träskman-Benz, L. (2004) Impulsivity related to brain serotonin transporter binding capacity in suicide attempters. *Eur. Neuropsychopharmacol.*, 14(4): 295–300.
- Lowther, S., de Parmentier, F., Cheetham, S., Crompton, M., Katona, C. and Horton, R. (1997) 5-HT_{1A} receptor binding sites in post-mortem brain samples from depressed suicides and controls. *J. Affect. Disord.*, 42(2–3): 199–207.
- Malick, J.B. and Barnett, A. (1976) The role of serotonergic pathways in isolation-induced aggression in mice. *Pharmacol. Biochem. Behav.*, 5(1): 55–61.
- Mann, J.J. (2003) Neurobiology of suicidal behavior. *Nat. Rev. Neurosci.*, 4(10): 819–828.
- Mann, J.J., Ellis, S.P., Waternaux, C.M., Liu, X., Oquendo, M.A., Malone, K.M., Brodsky, B.S., Haas, G.L. and Currier, D. (2008) Classification trees distinguish suicide attempters in major psychiatric disorders: a model of clinical decision making. *Clin. Psychiatry*, 69(1): 23–31.
- Mann, J.J., Hyang, Y.Y., Underwood, M.D., Kassir, S.A., Oppenheim, S., Kelly, T.M., Dwork, A.J. and Arango, V. (2000) A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch. Gen. Psychiatry*, 57(8): 729–738.
- Mann, J.J., McBride, P.A., Brown, R.P., Linnoila, M., Leon, A.C., DeMeo, M., Mieczkowski, T., Myers, J.E. and Stanley, M. (1992) Relationship between central and peripheral serotonin indexes in depressed and suicidal psychiatric inpatients. *Arch. Gen. Psychiatry*, 49(6): 443–446.
- Mann, J.J., Waternaux, C., Haas, G.L. and Malone, K.M. (1999) Towards a clinical model of suicidal behavior in psychiatric patients. *Am. J. Psychiatry*, 156(2): 181–189.
- McCloskey, M.S., Ben-Zeev, D., Lee, R. and Coccaro, E.F. (2008) Prevalence of suicidal and self-injurious behavior among subjects with intermittent explosive disorder. *Psychiatry Res.*, 158(2): 248–250.
- McGirr, A., Renaud, J., Bureau, A., Seguin, M., Lesage, A. and Turecki, G. (2007) Impulsive aggressive behaviours and completed suicide across the life cycle: a predisposition for younger age of suicide. *Psychol. Med.*, 38(3): 407–417.
- Meyer, J.H., Houle, S., Sagrati, S., Carella, A., Hussey, D.F., Ginovart, N., Goulding, V., Kennedy, J. and Wilson, A.A. (2004) Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch. Gen. Psychiatry*, 61(12): 1271–1279.
- Meyer, J.H., McNeely, H.E., Sagrati, S., Boovariwala, A., Martin, K., Verhoeff, N.P., Wilson, A.A. and Houle, S. (2006) Elevated putamen D(2) receptor binding potential in major depression with motor retardation: an [¹¹C]raclopride positron emission tomography study. *Am. J. Psychiatry*, 163(9): 1594–1602.
- Miczek, K.A. and de Almeida, R.M. (2001) Oral drug self-administration in the home cage of mice: alcohol-heightened aggression and inhibition by the 5-HT_{1B} agonist anpirtoline. *Psychopharmacology (Berl.)*, 157(4): 421–429.
- Montgomery, A.J., Stokes, P., Kitamura, Y. and Grasby, P.M. (2007) Extrastriatal and striatal D2 receptors in depressive illness: pilot PET studies using [¹¹C]FLB 457 and [¹¹C]raclopride. *J. Affect. Disord.*, 101(1–3): 113–122.
- Montgomery, S.A. and Åsberg, M. (1979) A new depression scale designed to be sensitive to change. *Br. J. Psychiatry*, 134: 382–389.
- Neves, F.S., Silveira, G., Romano-Silva, M.A., Malloy-Diniz, L., Ferreira, A.A., De Marco, L. and Correa, H. (2008) Is the 5-HTTLPR polymorphism associated with bipolar disorder or with suicidal behavior of bipolar disorder patients? *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 147(1): 114–116.
- O'Donnell, L., Stueve, A. and Wilson-Simmons, R. (2005) Aggressive behaviors in early adolescence and subsequent suicidality among urban youths. *J. Adolesc. Health*, 37(6): p. 517.
- Oreland, L., Wiberg, A., Åsberg, M., Träskman, L., Sjöstrand, L., Thorén, P., Bertilsson, L. and Tybring, G. (1981) Platelet MAO activity and monoamine metabolites in cerebrospinal fluid in depressed and suicidal patients and in healthy controls. *Psychiatry Res.*, 4(1): 21–29.
- Owen, F., Chambers, D.R., Cooper, S.J., Crow, T.J., Johnson, J.A., Lofthouse, R. and Poulter, M. (1986) Serotonergic mechanisms in brains of suicide victims. *Brain Res.*, 362(1): 185–188.
- Parsey, R.V., Hastings, R.S., Oquendo, M.A., Huang, Y.Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R.T., Van Heertum, R.L., Arango, V. and Mann, J.J. (2006) Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am. J. Psychiatry*, 163(1): 52–58.
- Pitchot, W., Hansenne, M. and Ansseau, M. (2001a) Role of dopamine in non-depressed patients with a history of suicide attempts. *Eur. Psychiatry*, 16(7): 424–427.
- Pitchot, W., Hansenne, M., Gonzalez Moreno, A., Pinto, E., Reggers, J., Fuchs, S., Pirard, S. and Ansseau, M. (2001b) Reduced dopamine function in depressed patients is related to suicidal behavior but not its lethality. *Psychoendocrinology*, 26(7): 689–696.
- Pitchot, W., Reggers, J., Pinto, E., Hansenne, M. and Ansseau, M. (2003) Catecholamine and HPA axis dysfunction in depression: relationship with suicidal behavior. *Neuropsychobiology*, 47(3): 152–157.
- Pitchot, W., Reggers, J., Pinto, E., Hansenne, M., Fuchs, S., Pirard, S. and Ansseau, M. (2001c) Reduced dopaminergic

- activity in depressed suicides. *Psychoendocrinology*, 26(3): 331–335.
- Roy, A. (1982) Risk factors for suicide in psychiatric patients. *Arch. Gen. Psychiatry*, 39(9): 1089–1095.
- Ryding, E., Ahnlied, J.-A., Lindström, M., Rosén, I. and Träskman-Bendz, L. (2006) Regional brain serotonin and dopamine transporter binding capacity in suicide attempters relate to impulsiveness and mental energy. *Psychiatry Res.*, 148(2–3): 195–203.
- Sher, L., Oquendo, M.A., Galfalvy, H.C., Grunebaum, M.F., Burke, A.K., Zalsman, G. and Mann, J.J. (2005) The relationship of aggression to suicidal behavior in depressed patients with a history of alcoholism. *Addict. Behav.*, 30(6): 1144–1153.
- Shih, J.C., Chen, K. and Ridd, M.J. (1999) Monoamine oxidase: from gene to behaviour. *Annu. Rev. Neurosci.*, 22: 197–217.
- Simon, G.E. and Savarino, J. (2007) Suicide attempts among patients starting depression treatment with medications or psychotherapy. *Am. J. Psychiatry*, 164(7): 1029–1034.
- Skondras, M., Markianos, M., Botsis, A., Bistolaki, E. and Christodoulou, G. (2004) Platelet monoamine oxidase activity and psychometric correlates in male violent offenders imprisoned for homicide or other violent acts. *Eur. Arch. Psychiatry Clin. Neurosci.*, 254(6): 380–386.
- Smith, K. and Crawford, S. (1986) Suicidal behavior among “normal” high school students. *Suicide Life Threat. Behav.*, 16(3): 313–325.
- Soderstrom, H., Blennow, K., Manhem, A. and Forsman, A. (2001) CSF studies in violent offenders. 5-HIAA as a negative and HVA as a positive predictor for psychopathy. *J. Neural Transm.*, 108(7): 869–878.
- Spirito, A., Valeri, S., Boergers, J. and Donaldson, D. (2003) Predictors of continued suicidal behavior in adolescents following a suicide attempt. *J. Child Adolesc. Psychol.*, 32(2): 284–289.
- Stanley, M. and Mann, J.J. (1983) Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet*, 1(8318): 214–216.
- Telang, F.W., Volkow, N.D., Levy, A., Logan, J., Fowler, J.S., Felder, C., Wong, C. and Wang, G.J. (1999) Distribution of tracer levels of cocaine in the human brain as assessed with averaged [^{11}C]cocaine images. *Synapse*, 31(4): 290–296.
- Tiihonen, J., Kuoppamäki, M., Nägren, K., Bergman, J., Eronen, E., Syvälahti, E. and Hietala, J. (1996) Serotonergic modulation of striatal D2 dopamine receptor binding in humans measured with positron emission tomography. *Psychopharmacology*, 126(4): 277–280.
- Träskman, L., Asberg, M., Bertilsson, L. and Sjöstrand, L. (1981) Monoamine metabolites in CSF and suicidal behavior. *Arch. Gen. Psychiatry*, 38(6): 631–636.
- Träskman-Bendz, L. and Mann, J. (2000) Biological aspects of suicidal behavior. In: Hawton K. and van Heeringen K. (Eds.), *The International Handbook of Suicide and Attempted Suicide*. Wiley, Chichester, UK, pp. 65–77.
- Tremblay, P.F., Mihic, L., Graham, K. and Jelly, J. (2007) Role of motivation to respond to provocation, the social environment, and trait aggression in alcohol-related aggression. *Aggress. Behav.*, 33(5): 389–411.
- Tupala, E. and Tiihonen, J. (2004) Dopamine and alcoholism: neurological basis of ethanol abuse. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28(8): 1221–1247.
- Tureki, G. (2005) Dissecting the suicide phenotype: the role of impulsive-aggressive behaviors. *Psychiatry Neurosci.*, 30(6): 398–408.
- Valzelli, L. (1982) Serotonergic inhibitory control of experimental aggression. *Pharmacol. Res. Commun.*, 14(1): 1–13.
- van Erp, A.M. and Miczek, K.A. (2000) Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *J. Neurosci.*, 20(24): 9320–9325.
- van Erp, A.M. and Miczek, K.A. (2007) Increased accumbal dopamine during daily alcohol consumption and subsequent aggressive behavior in rats. *Psychopharmacology (Berl.)*, 191(3): 679–688.
- van Heeringen, C., Audenaert, K., van Laere, K., Dumont, F., Slegers, G., Mertens, J. and Dierckx, R. (2003) Prefrontal 5-HT $_{2a}$ receptor binding index, hopelessness and personality characteristics in attempted suicide. *J. Affect. Disord.*, 74(2): 149–158.
- Verkes, R.J., Van der Mast, R.C., Kerkhof, A.J., Fekkes, D., Hengeveld, M.W., Tuyl, J.P. and Van Kempen, G.M. (1998) Platelet serotonin, monoamine oxidase activity, and [^3H] paroxetine binding related to impulsive suicide attempts and borderline personality disorder. *Biol. Psychiatry*, 43(10): 740–746.
- Wasserman, D., Geijer, T., Sokolowski, M., Frisch, A., Michaelovsky, E., Weizman, A., Rozanov, V. and Wasserman, J. (2007) Association of the serotonin transporter promoter polymorphism with suicide attempters with a high medical damage. *Eur. Neuropsychopharmacol.*, 17(3): 230–233.
- Willeit, M., Praschak-Rieder, N., Neumeister, A., Pirker, W., Asenbaum, S., Vitouch, O., Tauscher, J., Hilger, E., Stastny, J., Brücke, T. and Kasper, S. (2000) [^{123}I]-beta-CIT SPECT imaging shows reduced brain serotonin transporter availability in drug-free depressed patients with seasonal affective disorder. *Biol. Psychiatry*, 47(6): 482–489.
- Zalsman, G., Anderson, G.M., Peskin, M., Frisch, A., King, R.A., Vekslerchik, M., Sommerfeld, E., Michaelovsky, E., Sher, L., Weizman, A. and Apter, A. (2005) Relationships between serotonin transporter promoter polymorphism, platelet serotonin transporter binding and clinical phenotype in suicidal and non-suicidal adolescent inpatients. *J. Neural Transm.*, 112(2): 309–315.
- Zalsman, G., Huang, Y.Y., Oquendo, M.A., Burke, A.K., Hu, X.Z., Brent, D.A., Ellis, S.P., Goldman, D. and Mann, J.J. (2006) Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *Am. J. Psychiatry*, 163(9): 1588–1593.
- Zonda, T. (2006) One hundred cases of suicide in Budapest: a case-controlled psychological autopsy study. *Crisis*, 27(3): 125–129.
- Zouk, H., McGirr, A., Lebel, V., Benkelfat, C., Roleau, G. and Turecki, G. (2007) The effect of genetic variation of the serotonin 1B receptor gene on impulsive aggressive behavior and suicide. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 144(8): 996–1002.

CHAPTER 16

Prospects for serotonin 5-HT₂R pharmacotherapy in psychostimulant abuse

Marcy J. Bubar and Kathryn A. Cunningham*

Center for Addiction Research, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-0616, USA

Abstract: The serotonin (5-HT) neurotransmitter system provides fundamental modulatory regulation of the limbic-corticostriatal circuitry known to be vital in the development of addiction as well as the aspects of addiction that hinder recovery and contribute to relapse. Thus, components of the 5-HT system may provide novel targets for the development of pharmacological treatments for psychostimulant dependence, which is associated with significant aberrations in dopamine (DA) neurotransmission. Two key modulators of DA signalling within the limbic-corticostriatal circuit are the 5-HT_{2A} receptor (5-HT_{2A}R) and the 5-HT_{2C}R. These receptors are known to control the neurochemical and behavioural effects of psychostimulants, and in particular the in vivo effects of cocaine. Pre-clinical studies indicate that 5-HT_{2A}R antagonists and/or 5-HT_{2C}R agonists may effectively reduce craving and/or relapse, and likewise, enhance abstinence, while 5-HT_{2C}R agonists may also effectively reduce cocaine intake in active cocaine users. At present, the progression of studies to probe the effectiveness of 5-HT_{2A}R and 5-HT_{2C}R ligands in the clinical setting is hindered by a lack of available, selective 5-HT_{2A}R antagonists or 5-HT_{2C}R agonists for use in human cocaine abusers. However, a number of selective 5-HT₂R ligands currently under development, or in early clinical trials for psychiatric and/or neurological disorders, may soon be available for translational studies to explore their effectiveness in modulating drug use and dependence.

Keywords: 5-HT_{2A} receptor; 5-HT_{2C} receptor; cocaine; animal models; pharmacotherapy

Introduction

Substance abuse and dependence continue to plague society, with an estimated economic cost of over \$245 billion annually (Office of National Drug Control Policy, 2001). Addiction is a chronic disorder punctuated by multiple remissions and relapses. Pharmacotherapeutic approaches to maintain recovery in addicts are available and can

be effective in reducing 'craving' (desire for drug) and drug seeking, primarily in nicotine, alcohol and heroin users (for review, see O'Brien, 2005). However, approximately 60% of alcohol-, opioid- or cocaine-dependent patients who received treatment medications relapsed into drug use within 1 year (McLellan et al., 1996, 2000). Furthermore, only 8.5% of those who needed treatment of drug abuse and addiction received treatment in 2005, with cost and inaccessibility cited as barriers (Substance Abuse and Mental Health Services Administration, 2005). Thus, validation of new medications is an important step in developing a

*Corresponding author. Tel.: +409-772-9629;
Fax: +409-772-9642; E-mail: kcunning@utmb.edu

larger toolbox of effective treatment modalities for addiction.

The dopamine (DA) neurotransmitter system has been the primary focus of studies on the mechanisms underlying addiction as well as a key target for the development of pharmacotherapeutic agents for the treatment of substance use disorders (for review,

see [Vocci et al., 2005](#)). This focus has been justifiably driven by the key role for DA in the rewarding aspects of abused drugs. The DA mesoaccumbens pathway, originating in DA somata of the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAc; see [Fig. 1](#)), mediates the reinforcing effects of natural rewards (i.e. food and

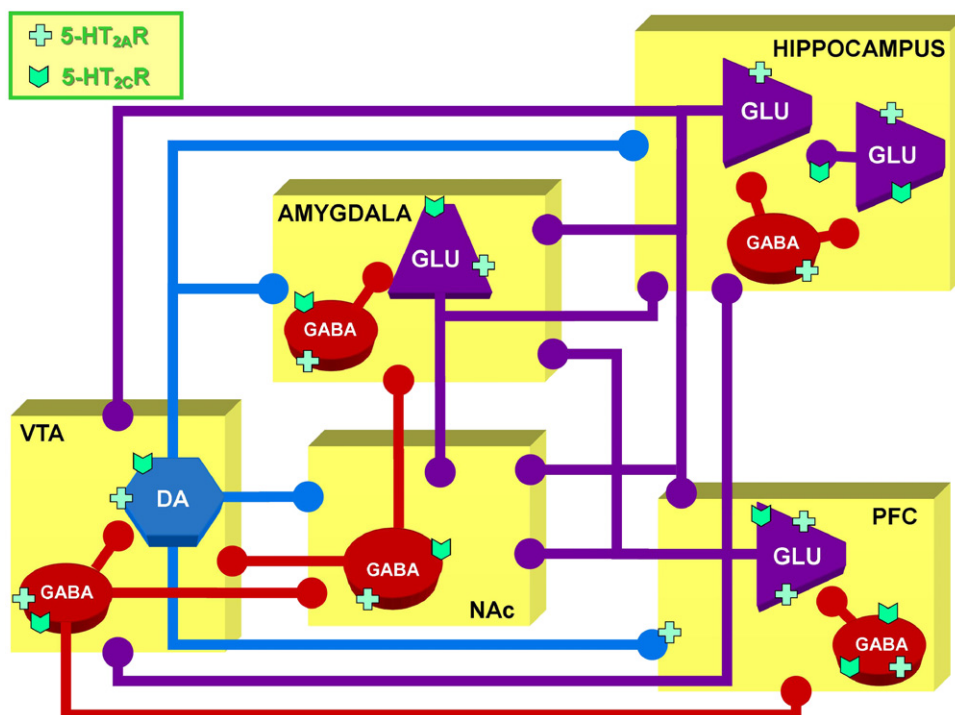


Fig. 1. (grayscale) Localization of 5-HT₂R in the limbic-cortico-striatal circuit. Schematic representation of the multitude of sites of action for the 5-HT_{2A}R (plus symbol) and 5-HT_{2C}R (chevron) to modulate neurotransmission within the limbic-cortico-striatal circuit. The diagram shows DA (black), GABA (light gray) and glutamate (dark gray) neuronal projections within five nodes of the circuit: the VTA, NAc, PFC, amygdala, and hippocampus. 5-HT neurons from the dorsal raphe nucleus innervate all five brain areas (5-HT pathways not shown). DA neurons in the VTA project to the NAc, PFC, amygdala and hippocampus. GABA neurons in the VTA synapse locally to modulate VTA DA neurons and also project to the NAc and PFC. GABA medium spiny neurons in the NAc innervate the VTA and amygdala in addition to other primary systems (e.g., striato-pallido-thalamic circuit; not shown). GABA interneurons localized to the PFC, amygdala and hippocampus modulate excitatory transmission of pyramidal glutamate neurons that project from the PFC to the VTA, NAc, amygdala and hippocampus; from the amygdala to the NAc and hippocampus; and from the hippocampus to the VTA, NAc, PFC and amygdala. Pyramidal glutamate neurons in the CA1 region of the hippocampus project locally to synapse upon pyramidal glutamate projection neurons located in the CA3 region. Details and references for 5-HT₂R localization within the limbic-cortico-striatal circuit are provided in the text. Briefly, the 5-HT_{2A}R and 5-HT_{2C}R are localized to both DA and GABA neurons in the VTA, although subtle differences in distribution are evident, and both receptors are localized to GABA medium spiny neurons in the NAc. In the PFC, the 5-HT_{2A}R is predominantly localized to pyramidal glutamate neurons, with lesser expression in GABA interneurons and dopaminergic terminals. The 5-HT_{2C}R, on the other hand, is predominantly localized to GABA interneurons in the PFC, with lesser expression in pyramidal glutamate neurons in this region. In the amygdala, 5-HT_{2A}R and 5-HT_{2C}R are localized on both GABA interneurons and pyramidal glutamate projection neurons. The 5-HT_{2A}R in the hippocampus is localized to CA1 and CA3 pyramidal neurons as well as GABA interneurons, while the 5-HT_{2C}R is thought to be localized to cell bodies and potentially presynaptic terminals of CA3 pyramidal neurons that synapse locally upon CA1 pyramidal glutamate projection neurons. (See Color Plate 16.1 in color plate section.)

sex; for review, see Kelley and Berridge, 2002) and also serves as a common substrate for the acute rewarding effects of all drugs of abuse regardless of their individual mechanisms of action (for reviews, see Koob and Le, 2001; Nestler, 2001, 2005). In addition, dopaminergic influence, in conjunction with glutamatergic neurotransmission, within nodes of the limbic-corticostratial circuitry [prefrontal cortex (PFC), amygdala, hippocampus; see Fig. 1] is integral in coordinating reward-related associative learning and motivated behaviours that contribute to aspects of addiction such as craving, withdrawal and relapse (for reviews, see Kalivas and Volkow, 2005; Hyman et al., 2006; Kauer and Malenka, 2007). Despite the undisputed evidence for the importance of DA in mediating addictive processes associated with drug use, dopaminergic ligands have not proven optimal as successful therapeutic medications in addiction. This is likely because pharmacological manipulations of DA systems interfere with normal physiological and behavioural function subserved by this system, and the use of DA ligands is associated with a myriad of negative side effects (Donna et al., 2002; Soares et al., 2003).

The serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system has been shown to provide tonic and phasic control of DA and glutamate neurotransmission within the limbic-corticostratial reward pathway (for review, see Alex and Pehek, 2007), and, as such, has become a favourable target for novel strategies for development of pharmacotherapeutics for addiction. 5-HT neurons originate in the raphe nuclei in the midbrain and project to numerous regions throughout the brain (Halliday and Tork, 1989), including a dense innervation of terminals to the VTA, NAc, PFC, amygdala, hippocampus and dorsal striatum (Halliday and Tork, 1989). The actions of 5-HT are mediated through at least 16 receptor subtypes (5-HTRs) grouped into seven families (5-HT₁R–5-HT₇R) according to their structural and functional characteristics, and include 13 distinct G-protein coupled receptors, coupled to various effector systems, and three ligand-gated ion channels (the 5-HT₃R) (for reviews, see Hoyer et al., 2002; Green, 2006). The 5-HTRs therefore provide a diverse landscape of available signalling cascades and mechanisms

unparalleled in any other neurotransmitter system. As such, a plethora of potential sites of action exist which may be integral for the integration of 5-HT, with signalling important to the impact of abused drugs on brain function.

The serotonin reuptake transporter (SERT) and the 5-HTRs are important sites of action for medications therapeutically effective in multiple psychiatric disorders (e.g. anxiety, depression, schizophrenia) and physiological disorders (e.g. migraine, irritable bowel syndrome) (for review, see Naughton et al., 2000; Jones and Blackburn, 2002). Growing evidence (Ait-Daoud et al., 2006; Bubar and Cunningham, 2006; Rothman et al., 2006; Sekine et al., 2006; El-Mallakh and Abraham, 2007; Hughes et al., 2007; Levin and Rezvani, 2007; Moeller et al., 2007; Tambour and Quertemont, 2007; Vocci and Appel, 2007) also supports the prospects of therapeutic gains for serotonergic medications in alcohol and drug abuse disorders. Much of this research has focused on the utility for treatment of psychostimulant addicts because there are currently no medications for successfully maintaining recovery from psychostimulant addiction. In particular, a large body of pre-clinical evidence exists that describes an integral role for 5-HT modulation of the neurochemical and behavioural effects of cocaine.

The psychostimulant cocaine, the alkaloid of *Erythroxylum coca*, binds to the SERT, inhibiting 5-HT reuptake and increasing synaptic 5-HT efflux in addition to its similar actions at DA and norepinephrine transporters (Koe, 1976). Pharmacological and genetic manipulations of 5-HT neurotransmission demonstrate roles for several of the 16 distinct 5-HTRs in the control of cocaine-induced behaviours (Cunningham and Callahan, 1994; Callahan and Cunningham, 1995, 1997; De La Garza et al., 1996, 1998; Walsh and Cunningham, 1997; McCreary and Cunningham, 1999; McMahon and Cunningham, 1999, 2001a; De La Garza and Cunningham, 2000; McMahon et al., 2001; Filip and Cunningham, 2002, 2003; Filip et al., 2004, 2006; Frankel and Cunningham, 2004; Szucs et al., 2005; Liu and Cunningham, 2006; Navailles et al., 2008). The 5-HT₂R family is particularly interesting as a target for development of therapeutic medications (Higgins and Fletcher, 2003; De La

Garza et al., 2005; Bubar and Cunningham, 2006; Muller and Carey, 2006). We will review the evidence in support of a role for the 5-HT_{2A}R and 5-HT_{2C}R based on pre-clinical studies.

Distribution of 5-HT_{2A}R and 5-HT_{2C}R

The 5-HT₂R subfamily of seven transmembrane region G-protein-coupled 5-HTRs is composed of three proteins: the 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R, derived from three different genes. The 5-HT_{2B}R protein, expressed primarily in the periphery (Kursar et al., 1994; Bonhaus et al., 1995), is thought to play a negligible role in the central effects of drugs of abuse (Bankson and Cunningham, 2002; Fletcher et al., 2002a, b; Filip et al., 2004, 2006) and, as such, will not be discussed here. However, it should be noted that due to the peripheral actions of the 5-HT_{2B}R, an important consideration for minimizing potential side effects when developing novel therapeutic medications for addiction is the selectivity of a ligand versus the 5-HT_{2B}R, given that chronic agonist activation at the 5-HT_{2B}R is identified as detrimental to cardiac valves (Setola et al., 2005; Kaumann and Levy, 2006).

The 5-HT_{2A}R and 5-HT_{2C}R are widely expressed throughout the brain (Pompeiano et al., 1994), and concordance of mRNA and protein expression (Mengod et al., 1990a, b; Pompeiano et al., 1994; Burnet et al., 1995; Wright et al., 1995; Lopez-Gimenez et al., 2001a, b) suggests predominant postsynaptic localization of these receptors, although presynaptic localization of 5-HT_{2A}R and 5-HT_{2C}R may also exist in some brain areas (Mengod et al., 1990a, b; Pompeiano et al., 1994; Lopez-Gimenez et al., 2001a). Although the 5-HT_{2C}R transcript is more abundant and is expressed in a greater number of brain areas than is the 5-HT_{2A}R (Pompeiano et al., 1994; Wright et al., 1995), the 5-HT_{2A}R and 5-HT_{2C}R coexist in several brain areas (Pompeiano et al., 1994), including the brain regions associated with the limbic-corticostriatal circuits (VTA, NAc, PFC, amygdala, hippocampus, dorsal striatum).

Knowledge of the sites of action of the 5-HT_{2A}R and 5-HT_{2C}R within the limbic-corticostriatal

circuits (see Fig. 1) can provide much needed insight into the potential mechanisms by which the receptors exert their actions. Several recent studies have closely examined the distribution of the 5-HT_{2A}R and 5-HT_{2C}R in the VTA, a complex structure composed of five subnuclei that differ in their afferent and efferent projections (Phillipson, 1979; Swanson, 1982). The 5-HT_{2A}R (Doherty and Pickel, 1999; Ikemoto et al., 2000; Nocjar et al., 2002) and the 5-HT_{2C}R (Bubar and Cunningham, 2005, 2007) proteins were found to be localized to both of the major neuronal populations in the VTA, DA and γ -aminobutyric acid (GABA) neurons. Although the explicit rostrocaudal and subnuclei patterns of colocalization of the 5-HT_{2A}R to non-dopaminergic (presumably GABA) VTA neurons have not yet been described, 5-HT_{2C}R mRNA and protein expression in VTA GABA neurons was found to be relatively uniform across the rostrocaudal gradient and among the VTA subnuclei [parabrachial pigmented nucleus (PBP), paranigral nucleus (PN), interfascicular nucleus (IF), rostral linear nucleus and caudal linear raphe nucleus] (Eberle-Wang et al., 1997; Bubar and Cunningham, 2007). Conversely, the two receptors were shown to be differentially expressed in VTA DA neurons along distinct rostrocaudal and subnuclear patterns (Doherty and Pickel, 1999; Ikemoto et al., 2000; Nocjar et al., 2002; Bubar and Cunningham, 2007). The greatest colocalization of 5-HT_{2A}R in DA neurons were found in the rostral and middle levels of the PN, the middle PBP and the IF (Nocjar et al., 2002). The 5-HT_{2C}R, however, was found to be most highly expressed in the middle levels of the PN, PBP and IF, as well as the rostral PBP (Bubar and Cunningham, 2007). While the functional significance of these distinct expression patterns is not fully understood at this time, these data indicate multidimensional opportunities for 5-HT_{2A}R and 5-HT_{2C}R to discretely modulate function of important DA pathways that originate in the VTA (Fig. 1).

By and large, the subcellular distribution of the 5-HT_{2A}R within the remainder of the limbic-corticostriatal circuit has been more thoroughly described than the distribution of the 5-HT_{2C}R. In the NAc, protein for the 5-HT_{2A}R is localized to

GABA medium spiny projection neurons in both the core and shell (Cornea-Hebert et al., 1999) (Fig. 1). The mRNA for the 5-HT_{2C}R was detected in what appeared to be GABA medium spiny projection neurons in both the core and shell of the NAc, with decreasing rostrocaudal gradient (Eberle-Wang et al., 1997). The 5-HT_{2A}R protein in the cortex is most highly expressed in layer V cortical pyramidal (glutamate) neurons, particularly within the apical dendritic field; some monoamine (presumably DA) nerve terminals as well as large- and medium-sized GABA interneurons in the cortex were also found to express 5-HT_{2A}R (Cornea-Hebert et al., 1999; Jakab and Goldman-Rakic, 2000; Miner et al., 2003). These GABA neurons typically synapse upon (Conde et al., 1990) and regulate firing of cortical pyramidal neurons (Gonzalez-Burgos et al., 2005). Conversely, the 5-HT_{2C}R mRNA was also detected in medium-sized GABA interneurons in layer V of the PFC (Pasqualetti et al., 1999); only low levels of expression of 5-HT_{2C}R mRNA was observed in cortical pyramidal neurons (Lopez-Gimenez et al., 2001a). In keeping with this finding, our laboratory recently demonstrated that 5-HT_{2C}R protein was predominantly expressed within parvalbumin-containing GABA interneurons localized to the deep layers (V/VI) of the PFC (Liu et al., 2007).

The expression of 5-HT_{2A}R protein in the amygdala was greatest in the basolateral amygdala in which expression was detected in both pyramidal cells and parvalbumin-containing GABA interneurons (McDonald and Mascagni, 2007). High levels of 5-HT_{2C}R mRNA were detected in 'large cells' in the dorsomedial amygdala (Pasqualetti et al., 1999). Electrophysiology experiments indicate that the 5-HT_{2C}R is localized to GABA inhibitory interneurons as well as excitatory glutamate neurons within the basolateral amygdala (Stein et al., 2000).

Some of the most intense immunostaining for the 5-HT_{2A}R in the brain was detected in the hippocampus (Cornea-Hebert et al., 1999); the 5-HT_{2A}R was expressed in the pyramidal cell layer (CA1-3) throughout the Ammon's horn and in the granular cell layer of the dentate gyrus, as well as in the majority of hippocampal GABA interneurons, suggesting that the 5-HT_{2A}R exerts direct and indirect modulation of hippocampal glutamatergic

cells (Luttgen et al., 2004). The 5-HT_{2C}R mRNA was expressed in a subset of pyramidal hippocampal cells restricted to the CA3 field of Ammon's horn (Pasqualetti et al., 1999), while 5-HT_{2C}R protein was detected in the pyramidal cell layer in both CA1 and CA3 (Clemett et al., 2000). Because the CA3 pyramidal cells project to the CA1, these data suggest that 5-HT_{2C}R protein in CA1 may be localized to presynaptic axon terminals from CA3 neurons (Pasqualetti et al., 1999).

Thus, the 5-HT_{2A}R and 5-HT_{2C}R are both prominently expressed in neurons throughout the limbic-corticostratial circuit (see Fig. 1), with distinct patterns of expression, thereby enabling differential modulation of neurotransmission by 5-HT_{2A}R and 5-HT_{2C}R localized within these circuits.

5-HT_{2A}R and 5-HT_{2C}R: functional regulation

The 5-HT_{2A}R and 5-HT_{2C}R display >50% overall amino acid sequence homology and >80% sequence homology within the transmembrane regions (Boess and Martin, 1994; Martin and Humphrey, 1994). Likewise, the 5-HT_{2A}R and 5-HT_{2C}R activate similar signalling pathways: each couple to G_{αq/11} to activate the downstream effector phospholipase C (PLC), promoting the hydrolysis of membrane phospholipids and the production of inositol-1,4,5-triphosphate and diacylglycerol leading to increased intracellular Ca²⁺ (Raymond et al., 2001; Hoyer et al., 2002). In addition, both receptors couple with G_{αq/11} to activate phospholipase A₂ (PLA₂) independent of PLC activation (Felder et al., 1990). PLA₂ releases arachidonic acid from the membrane, which directs a variety of actions including modulation of synaptic transmission, neurotransmitter uptake, protein kinase activity and ion channel activity (for review, see Katsuki and Okuda, 1995). Despite these similarities in signalling mechanisms, subtle differences between 5-HT_{2A}R and 5-HT_{2C}R signalling cascades also exist. For example, the 5-HT_{2C}R can also activate phospholipase D via G_{α13} and G_{βγ} subunits (McGrew et al., 2002). In addition, agonists have been shown to differentially activate signalling pathways (Berg et al., 1998)

and to couple to their effectors in an agonist-independent manner [constitutive activity; recently reviewed by Berg and colleagues (Berg et al., 2005)], although the degree of constitutive activity differs with regard to receptor and effector. For example, the 5-HT_{2C}R expresses up to 10 times greater constitutive activity towards PLC (determined by rate of phosphoinositide hydrolysis) than does the 5-HT_{2A}R (Teitler et al., 2002), but the 5-HT_{2C}R has relatively weak constitutive activity towards PLA₂ (determined by arachidonic acid release) (Berg et al., 1999). Recent studies have also found evidence for 5-HT_{2C}R homodimerization on the plasma membrane (Herrick-Davis et al., 2004, 2005), and endoplasmic reticulum (Herrick-Davis et al., 2006) of living cells suggesting that dimerization may also play an important role in the function of 5-HT_{2C}R. Although 5-HT_{2A}R homodimerization has not yet been described, functional studies implicate the involvement of 5-HT_{2C}R homodimerization in ligand binding, signal transduction and receptor trafficking processes (Herrick-Davis et al., 2004, 2005). The significance of constitutive activity and receptor dimerization in vivo has only recently come under investigation; thus, future investigations will provide a greater understanding of the importance of these processes in the regulation of 5-HT₂R function and the potential relevance for consideration in the development of pharmacotherapeutics.

One important mechanism of functional regulation that has obvious implications upon pharmacotherapeutic development is desensitization or down-regulation, observed as a decrease in responsiveness of the receptor, typically in response to agonist administration (for reviews, see Gray and Roth, 2001; Van Oekelen et al., 2003). Desensitization may occur via uncoupling of the receptor from its G-protein effector (Berg et al., 2001), sequestration of the receptor from the plasma membrane (internalization) (Saucier et al., 1998) and/or down-regulation of the receptor, as evidenced by a reduction in the number of binding sites (B_{\max}) due to lysosomal degradation and/or reduced mRNA or protein synthesis (Saucier et al., 1998; Gray et al., 2003). Indeed, both the 5-HT_{2A}R and 5-HT_{2C}R undergo rapid desensitization in response to agonist stimulation (Berg et al., 2001),

and prolonged agonist exposure induces down-regulation (Saucier et al., 1998; Willins et al., 1999; Tsao and von Zastrow, 2000; Gray et al., 2003; Van Oekelen et al., 2003; Schlag et al., 2004). In addition, both the 5-HT_{2A}R and 5-HT_{2C}R have also been shown to atypically down-regulate in response to repeated administration of ligands that act as antagonists at these receptors, although the mechanisms underlying this paradoxical response are not well understood (for reviews, see Gray and Roth, 2001; Van Oekelen et al., 2003). As such, the potential for receptor desensitization and down-regulation must be taken into account when evaluating potential 5-HT₂R ligands as treatment medications, particularly under conditions of repeated psychostimulant and ligand administration.

The 5-HT_{2A}R and 5-HT_{2C}R also undergo functionally relevant genetic and molecular events that result in the production of receptor variants. For example, both receptors undergo single nucleotide polymorphisms (SNPs) in which a single nucleotide (A, T, C, or G) in a DNA sequence is altered, resulting in protein variants that may differ in function, distribution or level of expression. Several polymorphisms in the 5-HT_{2A}R and 5-HT_{2C}R have been suggested to be important in controlling the response to treatment in psychiatric and neurological disorders (for reviews, see Parker et al., 2003; Reynolds et al., 2005; Serretti et al., 2007). In particular, the 5-HT_{2A}R T102C polymorphism has received attention for its involvement in the pharmacological response to atypical antipsychotics. Meta-analysis revealed a higher frequency of the T102C allele in patients who did not respond favourably to the atypical antipsychotic clozapine compared to those who responded well (Malhotra et al., 2004). The 5-HT_{2A}R T102C polymorphism was also found to be associated with impaired impulse control (Bjork et al., 2002), a trait correlated with substance dependence (Brady et al., 1998). Thus, these 5-HTR gene polymorphisms result in phenotypic differences that may contribute to the susceptibility or resistance to the initial and continued neural effects of psychostimulants in humans (Kreek et al., 2005).

An additional regulatory mechanism unique to the 5-HT_{2C}R (among G-protein coupled

receptors) occurs via mRNA editing (Niswender et al., 1999), a form of post-transcriptional modification (for review, see Sanders-Bush et al., 2003). mRNA editing is an example of an epigenetic change in gene expression that is not associated with changes in the DNA code and enables organisms to respond appropriately to changing environments (Maydanovich and Beal, 2006). Editing of the 5-HT_{2C}R mRNA creates single amino acid substitutions at up to three sites in the second intracellular loop of the receptor, producing 14 functionally distinct receptor isoforms with varying pharmacological and regulatory properties (Niswender et al., 1999). Edited isoforms of the 5-HT_{2C}R display reduced agonist affinity and potency, lower levels of constitutive activity and lower G-protein coupling efficiency (Herrick-Davis et al., 1999; Price et al., 2001; Marion et al., 2004). Furthermore, regulation of 5-HT_{2C}R mRNA editing is sensitive to changes in synaptic concentrations of 5-HT (Gurevich et al., 2002; Iwamoto and Kato, 2002). In general, the degree of editing is associated with the degree of receptor stimulation. For example, depletion of 5-HT via repeated *p*-chlorophenylalanine (PCPA) administration increased the expression of 5-HT_{2C}R mRNA isoforms encoding receptors with higher sensitivity to agonist stimulation, while treatment with the non-selective 5-HT₂R agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) significantly increased expression of the fully or partially edited 5-HT_{2C}R mRNA isoforms with decreased agonist sensitivity (Gurevich et al., 2002). Thus, prolonged stimulation of the 5-HT_{2C}R causes alterations in 5-HT_{2C}R mRNA editing, resulting in the expression of mRNA isoforms encoding receptors that least efficiently activate G-proteins and exhibit reduced agonist-stimulated activity (Gurevich et al., 2002). Therefore, 5-HT_{2C}R mRNA editing provides a unique form of receptor diversity that adds an additional level of functional regulation for the 5-HT_{2C}R.

5-HT_{2A}R and 5-HT_{2C}R modulation of DA neurotransmission

An important characteristic for establishing the 5-HT_{2A}R and 5-HT_{2C}R as targets for

pharmacotherapeutic intervention for substance use disorders is the ability of these receptors to modulate DA neurotransmission within the limbic-corticostriatal circuit. Indeed, two recent reports have reviewed in detail how pharmacological manipulation of 5-HT_{2A}R and 5-HT_{2C}R alters DA neurotransmission (Alex and Pehek, 2007; Fink and Gothert, 2007). Extracellular single unit recordings of VTA DA neuron firing have demonstrated that systemic administration of the non-selective 5-HT₂R agonist DOI increased the firing rates of DA neurons, while microdialysis measurements of DA efflux in terminal regions revealed that systemic administration of DOI increased DA release in the PFC (Bortolozzi et al., 2005; Pehek et al., 2006). These effects were completely reversed by the selective 5-HT_{2A}R antagonist M100907 (Bortolozzi et al., 2005; Pehek et al., 2006). Interestingly, systemic administration of 5-HT_{2A}R antagonists alone did not alter the firing rates of VTA DA neurons (Di Giovanni et al., 1999; Bonaccorso et al., 2002; Porras et al., 2002), DA release in the NAc (Di Giovanni et al., 1999; Bonaccorso et al., 2002; Porras et al., 2002) or PFC (Bonaccorso et al., 2002; Pehek et al., 2006; but see Schmidt and Fadaye, 1995). Thus, the 5-HT_{2A}R does not appear to exert tonic influence upon DA neuronal firing or DA release in the NAc or PFC, but stimulation of the 5-HT_{2A}R with an exogenous agonist reveals an enhancement of DA neuronal output which is physiologically significant.

The 5-HT_{2C}R, however, appears to provide both tonic and phasic modulation of DA neurotransmission. Systemic administration of 5-HT_{2C}R agonists decreased (Di Giovanni et al., 2000; Di Matteo et al., 2000), while 5-HT_{2C}R antagonists increased, basal firing rates of VTA DA neurons (De Deurwaerdere and Spampinato, 1999; Di Giovanni et al., 1999; Di Matteo et al., 1999). Similarly, microdialysis studies in terminal areas of the VTA neurons revealed that systemic administration of 5-HT_{2C}R agonists decreased (Di Matteo et al., 1999; Di Giovanni et al., 2000), while 5-HT_{2C}R antagonists increased, DA efflux in the NAc (De Deurwaerdere and Spampinato, 1999; Di Giovanni et al., 1999; Di Matteo et al.,

1999). In contrast, only the 5-HT_{2C}R antagonist enhanced DA release in the PFC after systemic administration, while the 5-HT_{2C}R agonist was ineffective at altering basal DA release in the PFC (Pozzi et al., 2002). Overall, these results suggest that the 5-HT_{2C}R tonically inhibits DA mesocorticoaccumbens output. This tonic inhibition is likely a result of high levels of constitutive activity of the 5-HT_{2C}R as evidenced by the ability of the 5-HT_{2C}R antagonist SB 242084 to block both the inhibition of DA release in the NAc induced by the 5-HT_{2C}R agonist RO 60-0175 and the enhancement of DA release in the NAc induced by the 5-HT_{2C}R inverse agonist SB 206553 (De Deurwaerdere et al., 2004). Taken together, these data indicate that the 5-HT_{2A}R and 5-HT_{2C}R provide opposing stimulatory and inhibitory influence, respectively, upon DA mesocorticoaccumbens output.

5-HT_{2A}R and 5-HT_{2C}R modulation of psychostimulant-evoked DA neurotransmission

Ligands for the 5-HT_{2A}R and 5-HT_{2C}R have also been shown to modulate efflux of DA induced upon systemic administration of psychostimulants. Similar to the effects observed upon basal DA release, systemic administration of 5-HT_{2C}R antagonists enhanced cocaine-evoked DA release in the NAc (De Deurwaerdere et al., 2004). In contrast, systemic administration of the non-selective 5-HT_{2A/2C}R antagonist ketanserin or the selective 5-HT_{2A}R antagonist SR 46349B reduced cocaine-evoked (Broderick et al., 2004) or amphetamine-evoked DA release in the NAc (Auclair et al., 2004a), respectively. These data suggest that 5-HT_{2A}R and 5-HT_{2C}R also play important stimulatory and inhibitory roles, respectively, in modulating the impact of cocaine and other psychostimulants on DA output. As such, it is not surprising that these receptors modulate the various behavioural effects of psychostimulants that are thought to be primarily mediated by dopaminergic mechanisms of action.

5-HT_{2A}R and 5-HT_{2C}R modulation of psychostimulant-evoked behaviours

A number of animal models are commonly utilized to examine the neurobiological mechanisms underlying the effects of drugs of abuse during various stages of the cycle of addiction (see Fig. 2). The largest volume of data suggesting that neurotransmission through the 5-HT_{2A}R and 5-HT_{2C}R may modulate the behavioural effects of psychostimulants exists from studies examining the effects of 5-HT₂R ligands on the hypermotive, stimulus and reinforcing effects of cocaine in rodents (see Table 1 for summary). Thus, this section primarily focuses on results from these cocaine studies, although results with other drugs of abuse are included when available.

Locomotor hyperactivity

The ability of a drug to elicit locomotor hyperactivity generally correlates with its ability to enhance DA release in the NAc (for review, see Cunningham and Callahan, 1994). Likewise, the pattern for 5-HT_{2A}R and 5-HT_{2C}R to modulate psychostimulant-evoked hyperactivity aligns with their modulation of psychostimulant-evoked DA efflux. Studies employing selective 5-HT_{2A}R and 5-HT_{2C}R ligands present a relatively consistent pattern of effects such that acute systemic injections of selective 5-HT_{2A}R *antagonists* and 5-HT_{2C}R *agonists* block, while 5-HT_{2A}R *agonists* and 5-HT_{2C}R *antagonists* enhance, the hypermotive effects of cocaine at doses of the ligands that do not modify basal behaviour (McCreary and Cunningham, 1999; McMahon et al., 2001; Fletcher et al., 2002a; Filip et al., 2004; Filip et al., 2006). A similar pattern of 5-HT_{2A}R and 5-HT_{2C}R modulation of behaviours was also replicated for amphetamine (Schmidt et al., 1992; Sorensen et al., 1993; Fletcher et al., 2006), which reverses the DA reuptake transporter, and 3,4-methylenedioxymethamphetamine (MDMA; ecstasy), which reverses both the 5-HT and DA reuptake transporters (McCreary et al., 1999; Bankson and Cunningham, 2001, 2002; Fletcher et al., 2002b). Thus, it appears that once the 5-HT_{2A}R and 5-HT_{2C}R can be distinguished

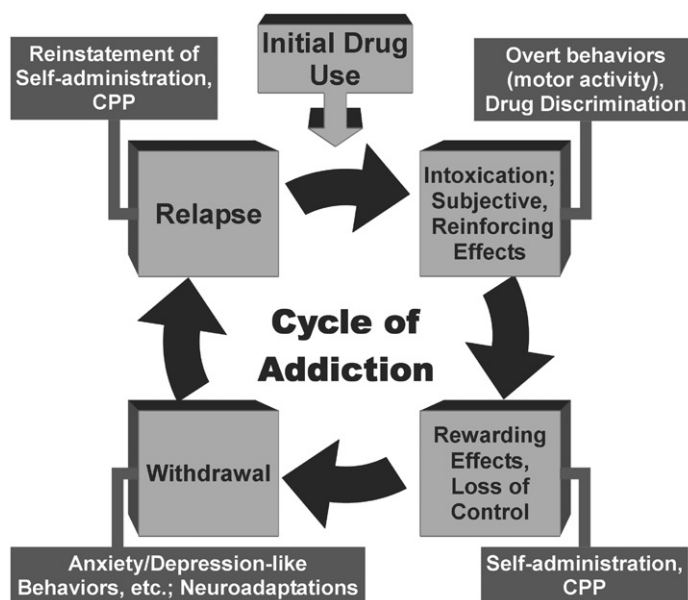


Fig. 2. Animal models of addiction. Schematic diagram displaying the stages within the cycle of addiction in humans and the animal models commonly used to study each stage. The cycle of addiction begins with the initial drug use, which is positively reinforced by the intoxicating and euphoric subjective effects of the drug (measured in animals by examining overt behaviours such as locomotor activity, and drug discrimination studies); these effects contribute to the reinforcing properties of the drugs which encourage an individual to take the drug again and again. Eventually, the rewarding effects of the drugs [measured in animals via self-administration and conditioned place preference (CPP)] become so intense that the individual loses control over their drug use to the point where they will continue to use the drug despite negative consequences. Users may eventually go through a period of withdrawal when they abstain from using the drug (in animals, studies at this stage typically measure anxiety- or depression-like states, or neuroadaptations that occur). However, this period of abstinence is typically followed by a relapse to drug use, which can be induced by exposure to stress, drug-related cues or the drug itself (measured in animals using reinstatement models of self-administration and CPP).

Table 1. 5-HT₂R ligands modulate the rodent behavioural response to cocaine

Behaviour	5-HT _{2A} R		5-HT _{2C} R	
	Agonist	Antagonist	Agonist	Antagonist
Hyperactivity	↑	↓	↓	↑
Drug discrimination	↑	↓	↓	↑
Self-administration		NE	↓	↑
Cocaine reinstatement		↓	↓	↑
Cue reinstatement		↓	↓	
CPP (acquisition, expression)		↓	↓	
Conditioned hyperactivity (expression)			↓	↑

↑, increase; ↓, decrease; NE, no effect; see text for references.

pharmacologically from one another, their influences include an excitatory role for the 5-HT_{2A}R and an inhibitory role for the 5-HT_{2C}R in the control of stimulant-induced behaviours.

Studies examining 5-HT₂R antagonist effects on hyperactivity induced by other psychoactive compounds revealed analogous patterns of modulation. Whereas 5-HT_{2A}R antagonists were shown to reduce the hyperactivity induced by morphine (Auclair et al., 2004b) and phencyclidine (PCP) (Maurel-Remy et al., 1995), a 5-HT_{2C}R antagonist enhanced hypermotility elicited by morphine, PCP and nicotine (Fletcher et al., 2006). These studies suggest that the ability of 5-HT₂R ligands to modulate hyperactivity is not limited to drugs that directly interact with the DA and/or 5-HT neurotransmitter systems, indicating that utility of these ligands in the treatment of substance use disorders could spread beyond psychostimulants.

5-HT_{2A}R and 5-HT_{2C}R sites of action within the mesocorticoaccumbens pathway

The almost perfect oppositional control of the hypermotive effects of psychostimulants attained upon systemic administration of 5-HT₂R ligands may be transduced through a variety of mechanisms. To explore the sites of action that contribute to these observed effects, our laboratory initiated studies to map sensitivity to 5-HT_{2A}R- and 5-HT_{2C}R-specific ligands microinjected into the various brain regions, beginning with nodes of the DA mesocorticoaccumbens pathway, the VTA, NAc or PFC. Consistent with the observation that systemic administration of 5-HT₂R antagonists alone have little effect on basal behaviours (McCreary and Cunningham, 1999; McMahon and Cunningham, 2001a; Bankson and Cunningham, 2002; Fletcher et al., 2002a, b; Filip et al., 2004, 2006), the microinjection of a selective 5-HT_{2A}R or 5-HT_{2C}R antagonist does not evoke overt behavioural effects at the doses infused into these nuclei (McMahon et al., 2001; Filip and Cunningham, 2002, 2003). However, in accordance with the overall effects of systemically administered 5-HT_{2A}R and 5-HT_{2C}R ligands on cocaine-evoked behaviours, regionally dependent

effects of the 5-HT₂R ligands were observed upon microinjection into the nuclei of the mesocorticoaccumbens pathway immediately before systemic cocaine injection.

Intra-VTA microinjection of the selective 5-HT_{2A}R antagonist M100907 blocked cocaine-evoked hyperactivity (McMahon et al., 2001) as well as the cFos expression (a marker of neuronal activation) in the shell of the NAc evoked by systemic administration of cocaine (Szucs et al., 2005). Intra-VTA infusion of another 5-HT_{2A}R antagonist, SR 46349B, was also shown to block amphetamine-induced hyperactivity as well as amphetamine-induced DA release in the NAc (Auclair et al., 2004a). Conversely, intra-NAc shell microinjection of M100907 had no effect on cocaine-evoked hyperactivity (McMahon et al., 2001), while intra-PFC infusion of SR 46349B was ineffective at altering amphetamine-evoked hyperactivity (Auclair et al., 2004a). These data suggest that 5-HT_{2A}R in the VTA plays a strategic role in mediating the hyperactivity induced by psychostimulants, likely via enhancing the degree of psychostimulant-evoked DA efflux in the NAc, while a contribution for the 5-HT_{2A}R in the NAc shell and PFC is not apparent at present.

A more complex picture for 5-HT_{2C}R modulation of cocaine-evoked hyperactivity is evident. While intra-VTA microinfusion of the 5-HT_{2C}R antagonist RS102221 was ineffective at altering cocaine-evoked hyperactivity (McMahon and Cunningham, 2001b; Filip and Cunningham, 2002), intra-VTA infusion of a 5-HT_{2C}R agonist blocked cocaine-evoked hyperactivity (Fletcher et al., 2004). These results suggest that the VTA 5-HT_{2C}R likely does not play an overt role in the control of cocaine-evoked hyperactivity. However, upon activation via selective 5-HT_{2C}R agonist administration, engagement of 5-HT_{2C}R-controlled pathways originating in the VTA limits the extent of hyperactivity evoked by cocaine (Fletcher et al., 2004). These results are supported by a recent study demonstrating that intra-VTA injection of the 5-HT_{2C}R agonist RO 60-0175 reduced the enhancement of DA outflow in the NAc induced by a systemic injection of cocaine, while intra-VTA administration of the 5-HT_{2C}R antagonist SB 242084 had no effect (Navailles

et al., 2008). These multifaceted effects could potentially be related to the level of constitutive activity of the 5-HT_{2C}R, the preponderance of partially to fully edited 5-HT_{2C}R isoforms expressed and/or the dynamic expression patterns of 5-HT_{2C}R protein within the VTA (Bubar et al., 2005; Bubar and Cunningham, 2007).

As opposed to the inhibitory effects of 5-HT_{2C}R agonists in the VTA, intra-NAc shell microinjection of the 5-HT_{2C}R antagonist RS 102221 attenuated, while intra-NAc shell infusion of either the 5-HT_{2C}R agonist MK 212 or RO 60-0175 enhanced, the hyperlocomotive effects of cocaine in a dose-related manner (McMahon and Cunningham, 2001b; Filip and Cunningham, 2002). These findings demonstrate that the behavioural effects of cocaine are generated, in part, by activation of 5-HT_{2C}R in the NAc shell, an effect opposite of that observed following systemic 5-HT_{2C}R ligand administration (Fletcher et al., 2002a; Filip et al., 2004). The functional importance of *excitatory* 5-HT_{2C}R in NAc, however, is consistent with the finding that intra-VTA infusion of a low concentration (0.1 µM) of RO 60-0175 increased, while the 5-HT_{2C}R antagonist SB 242084 decreased cocaine-evoked DA overflow in the NAc (Navailles et al., 2008). Interestingly, administration of a higher concentration (1 µM) of the 5-HT_{2C}R ligands revealed a biphasic response in which RO 60-0175 and SB 242084 decreased and increased, respectively, cocaine-evoked DA overflow in the NAc (Navailles et al., 2008), suggesting that 5-HT_{2C}R localized to the NAc are able to exert both inhibitory and excitatory control over the DA overflow in the NAc.

Intra-PFC microinjections of the 5-HT_{2C}R agonist MK 212 or the antagonist RS 102221 dose-dependently decreased or increased, respectively, the hyperlocomotive effects of cocaine (Filip and Cunningham, 2003), indicating that the 5-HT_{2C}R in the PFC exerts *inhibitory* control over the hypermotive effects of cocaine, an effect directionally identical with the influence of the 5-HT_{2C}R ligands after systemic administration. The results of the microinjection studies employing selective 5-HT_{2C}R ligands suggest a circumscribed role for 5-HT_{2C}R in the VTA and an opposing stimulatory and inhibitory influence of 5-HT_{2C}R

in the NAc and PFC, respectively, upon the hyperlocomotive effects of cocaine. These findings suggest that net influence of 5-HT_{2C}R on cocaine-evoked behaviours observed following systemic ligand administration results from a functional balance of influence from different populations of 5-HT_{2C}R localized to the VTA, NAc and PFC, with the population of the 5-HT_{2C}R in the PFC serving as a dominant influence.

In summary, the results of microinjection studies indicate that while the 5-HT_{2A}R and 5-HT_{2C}R in the mesocorticoaccumbens nuclei do not play an active, tonic role in behavioural control, separate populations of the 5-HT_{2A}R and 5-HT_{2C}R within the PFC, NAc and VTA differentially influence the output of the mesocorticoaccumbens DA pathway. The studies indicate that the VTA is one brain site in which the 5-HT_{2A}R provides excitatory influence upon cocaine-evoked behaviours, while the ventral PFC is a specific brain site at which the 5-HT_{2C}R exerts an inhibitory effect upon behavioural responses to cocaine (Filip and Cunningham, 2003; Liu et al., 2007). Further studies examining the contribution of the 5-HT_{2A}R and 5-HT_{2C}R located in brain areas outside the mesocorticoaccumbens circuit are necessary, however, to gain an overall understanding of the specific sites of action of these receptors throughout the brain.

Drug discrimination

The recognition of the 'interoceptive cue' or stimulus effects elicited by psychoactive drugs, as demonstrated using the drug discrimination assay in rodents, has proved to be useful to model the subjective effects of these drugs as described in humans. Once rodents have been trained to recognize the interoceptive state induced by an injection of a psychoactive drug (for review, see Callahan et al., 1997), the ability of neurotransmitter ligands to mimic, block or otherwise alter the expression of the stimulus effects of the drug in this assay provides insight into the mechanisms of action underlying the *in vivo* effects.

Studies employing selective 5-HT_{2A}R and 5-HT_{2C}R ligands present a similar pattern of effects in the cocaine drug discrimination assay as observed for psychostimulant-evoked hyperactivity,

such that acute systemic injections of selective 5-HT_{2A}R antagonists and 5-HT_{2C}R agonists block, while 5-HT_{2A}R agonists and 5-HT_{2C}R antagonists enhance the discriminative stimulus effects of cocaine (Callahan and Cunningham, 1995; McMahon et al., 2001; Munzar et al., 2002; Filip et al., 2006). In addition, microinjection studies again revealed a disparate influence of 5-HT_{2C}R ligands infused into the NAc and PFC on the discriminative stimulus effects of cocaine observed: Intra-NAc shell microinjection of the 5-HT_{2C}R antagonist RS 102221 attenuated the cocaine discriminative stimulus cue, while the 5-HT_{2C}R agonists MK 212 and RO 60-0175 enhanced the discriminability of submaximal doses of cocaine (Filip and Cunningham, 2002). Intra-PFC microinjections of the 5-HT_{2C}R agonist MK 212 or the antagonist RS 102221, however, dose-dependently decreased or increased, respectively, the discriminative stimulus effects of cocaine (Filip and Cunningham, 2003).

Agonists and antagonists at the 5-HT_{2A}R enhanced and blocked, respectively, the amphetamine (Moser et al., 1996; Marona-Lewicka and Nichols, 1997) and methamphetamine discriminative stimulus cues (Munzar et al., 1999). Conversely, both 5-HT_{2A}R and 5-HT_{2C}R agonists were recently shown to decrease the discriminative stimulus effects of nicotine (Quarta et al., 2007; Zaniewska et al., 2007), while 5-HT_{2A}R and 5-HT_{2C}R antagonists were without effect, suggesting that the 5-HT_{2A}R and 5-HT_{2C}R can both inhibit the nicotine stimulus cue, but are not required for its presentation (Zaniewska et al., 2007). Conversely, activation of the 5-HT_{2C}R mimics the ethanol discriminative stimulus cue, but 5-HT_{2A}R ligands were ineffective at altering the ethanol cue (Maurel et al., 1998). Thus, while the effects of 5-HT₂R ligands on the stimulus cues elicited by various psychostimulants appear to be similar (see Table 1), 5-HT_{2A}R and 5-HT_{2C}R have distinct influences upon other classes of abused drugs.

Self-administration and conditioned place preference

The quest to develop novel medications is reliant on behavioural assays that model aspects of the

complex phenomenon of drug-taking behaviour. The essential role of 'reward' in reinforcing drug-seeking behaviour has received considerable attention (Stolerman, 1993; Everitt and Wolf, 2002; Robinson and Berridge, 2003). Two experimental paradigms presently predominate as measures of cocaine reward: drug self-administration and conditioned place preference (CPP). Drug self-administration is solidly based upon the operant principle of 'reinforcement'; the presentation of a stimulus (e.g. cocaine as an appetitive 'reward') contingent upon a behavioural response increases the probability that that behaviour will reoccur. The CPP paradigm, however, is based upon the repeated pairing of a previously neutral context with cocaine; a CPP is evidenced by the increased time spent in the paired context relative to the unpaired context on a later drug-free test (Ettenberg, 1989). Thus, self-administration assays appear to be particularly useful for the study of mechanisms that underlie drug-taking and drug-seeking behaviour, while CPP is best geared to assess the mechanisms underlying the context-drug associative learning that is fundamental to the acquisition of the drug-reinforced operant response (Lu et al., 2003). A perfect concordance between drugs that are self-administered and those that support CPP is not found; however, investigations indicate that the receptor mechanisms that underlie these two behavioural measures are not identical (Bardo and Bevins, 2000), suggesting that self-administration and CPP are subserved by distinct neural circuitry (Bardo and Bevins, 2000). Thus, it is important to examine the effects of 5-HT₂R ligands on the rewarding and reinforcing properties of psychostimulants using both behavioural models in order to best understand the role of 5-HT_{2A}R and 5-HT_{2C}R in the processes that underlie 'rewarding' aspects of psychostimulants.

A differential influence of the 5-HT_{2A}R and 5-HT_{2C}R on the reinforcing efficacy of cocaine is revealed by results of self-administration assays. The selective 5-HT_{2A}R antagonist M100907 did not alter rates of responding for cocaine in the self-administration paradigm, suggesting that 5-HT_{2A}R plays little role in the rewarding effects of cocaine (Fletcher et al., 2002a). Similar results

were also observed in studies examining the effects of the non-selective 5-HT_{2A}R antagonist risperidone on amphetamine self-administration in rats (Brown et al., 1998), whereas blockade of the 5-HT_{2A}R by ketanserin and M100907 attenuated *S*(+)-MDMA self-administration and almost completely abolished the reinforcing effects of *R*(-)-MDMA in rhesus monkeys (Fantegrossi et al., 2002).

The selective 5-HT_{2C}R antagonist SB 242084 increased rates of cocaine self-administration in a dose-dependent manner (Fletcher et al., 2002a). Likewise, the selective 5-HT_{2C}R agonist reduced rates of cocaine self-administration (Grottick et al., 2000; Fletcher et al., 2004), suggesting that agonists for the 5-HT_{2C}R may reduce the reinforcing efficacy of cocaine. The 5-HT_{2C}R agonist also reduced responding for ethanol (Tomkins et al., 2002) and nicotine self-administration (Grottick et al., 2001). These studies therefore highlight 5-HT_{2C}R agonists as potential therapeutic agents in addicts by reducing the rewarding effects of cocaine and other drugs of abuse.

Although the 5-HT_{2A}R and 5-HT_{2C}R are thought to play an important role in the processes underlying the acquisition, consolidation and retrieval of learned responses (Meneses, 1999, 2002), the role of these receptors in the strong conditioned associations made between cocaine and environmental stimuli ('cues') is relatively unexplored. A limited number of genetic (Allan et al., 2001; Sora et al., 2001; Neumaier et al., 2002) and pharmacological manipulations (Harris et al., 2001) suggest that 5-HT is involved in the process of acquisition and/or expression of a cocaine CPP, although studies examining the influence of selective 5-HT_{2A}R and/or 5-HT_{2C}R ligands on cocaine CPP have yet to be published (Tzschentke, 2007). Preliminary data from our laboratory, however, indicate a prominent role for both the 5-HT_{2A}R and the 5-HT_{2C}R in cocaine CPP as the 5-HT_{2A}R antagonist and 5-HT_{2C}R agonist blocked both the acquisition and expression of single-trial cocaine CPP (Herin, dela Cruz and Cunningham, unpublished observations). In addition, studies using the cocaine-induced conditioned hyperactivity paradigm (i.e. measurement of hyperactivity induced by an environment

previously paired with cocaine) revealed that a 5-HT_{2C}R agonist reduced, while a 5-HT_{2C}R antagonist enhanced, cocaine-induced conditioned hyperactivity (Liu and Cunningham, 2006). These preliminary studies suggest that both the 5-HT_{2A}R and 5-HT_{2C}R may play roles in the conditioned associations made between cocaine and environmental cues and likewise warrant further examination into the modulatory effects of 5-HT₂R on cocaine CPP.

At the present time, animal models of relapse to drug-seeking behaviour are based predominantly on analyses of the resumption of the previous drug-reinforced behaviour upon the non-contingent exposure to the drug or to non-drug conditioned stimuli (de Wit and Stewart, 1981). Pharmacological means to reduce reinstatement of drug-lever responding are under study with the promise of serving as 'abstinence enhancers' (Katz and Higgins, 2003). Studies to date indicate that the neural mechanisms underlying cocaine-evoked versus conditioned cue-evoked reinstatement of drug-seeking behaviour may be differentiable (Kalivas and McFarland, 2003). Indeed, cocaine-evoked and conditioned cue-evoked reinstatements appear to be differentially affected by 5-HT₂R ligands. Although non-selective 5-HT₂R antagonists (Schenk, 2000; Burmeister et al., 2004) had no effect on cocaine-primed reinstatement, but depressed cue-evoked reinstatement (Burmeister et al., 2004), the acute administration of the selective 5-HT_{2A}R antagonist M100907, suppressed both cocaine-primed (Fletcher et al., 2002a) and cue-evoked reinstatement (Nic Dhonnchadha and Cunningham, unpublished observations). Cocaine-primed reinstatement was also blocked and enhanced by a 5-HT_{2C}R agonist and antagonist, respectively (Fletcher et al., 2002a, 2008; but see Burmeister et al., 2004; Neisewander and Acosta, 2007; Burbassi and Cervo, 2008), and the 5-HT_{2C}R agonist was shown to block cue-evoked (Fletcher et al., 2008) and stress-evoked reinstatement (Burmeister et al., 2004; Neisewander and Acosta, 2007; Burbassi and Cervo, 2008; Fletcher et al., 2008). The effects of the 5-HT_{2C}R agonist were reversed by administration of the selective 5-HT_{2C}R antagonist; however, the antagonist alone had no effect upon cue-evoked or stress-evoked

reinstatement of cocaine seeking (Willins et al., 1999; Tsao and von Zastrow, 2000; Gray et al., 2003; Van Oekelen et al., 2003; Schlag et al., 2004). These data suggest that antagonists at the 5-HT_{2A}R and agonists at the 5-HT_{2C}R reduce cocaine seeking, and therefore may be helpful in maintaining abstinence and reducing relapse in human cocaine abusers.

Interpretation of studies involving repeated psychostimulant or 5-HT₂R ligand administration must take into account the potential for functional regulation of the receptors. Neuroadaptations in the 5-HT_{2A}R, 5-HT_{2C}R and/or downstream signalling components consequent to the repeated exposure to the psychostimulant during self-administration could result in altered responsiveness to 5-HT₂R ligands. Certainly, both receptors demonstrate down-regulation following prolonged exposure to direct or indirect agonists (Darmani et al., 1990; Dworkin et al., 1995; Parsons et al., 1995), and 5-HT-regulated receptor desensitization has been suggested to play an important role in the response to cocaine (Fletcher et al., 2008). However, the ability of the 5-HT₂R to maintain their functional status upon repeated psychostimulant or 5-HT₂R ligand administration has not been thoroughly studied in behavioural assays. Fletcher and co-workers (Fletcher et al., 2008) recently reported that RO 60-0175 consistently blocked cocaine self-administration when administered repeatedly over a period of 8 days, suggesting that repeated administration of the agonist did not alter the functional ability of the 5-HT_{2C}R to modulate the effects of cocaine during repeated cocaine administration. Conversely, the effectiveness of a 5-HT_{2A}R agonist or 5-HT_{2C}R antagonist to enhance and a 5-HT_{2C}R agonist to block cocaine-evoked hyperactivity was eliminated at 5 days of withdrawal from a repeated intermittent regimen of cocaine, although the 5-HT_{2A}R antagonist retained its ability to block cocaine-evoked hyperactivity (Filip et al., 2004). The loss of effect of both the 5-HT_{2C}R agonist and antagonist following the repeated cocaine regimen suggests that the function of these receptors and/or their downstream signalling components may be compromised during withdrawal from repeated exposure to cocaine (Baumann and Rothman,

1998; Battaglia et al., 2000; Darmani and Ahmad, 2000; Yan et al., 2000; Carrasco and Battaglia, 2007). However, a number of studies indicate a transient supersensitivity of the 5-HT_{2A}R following single or repeated cocaine exposure (Carrasco et al., 2003, 2004), an effect that may be related to changes in expression of G_{αq/11} subunits rather than overt changes in 5-HT_{2A}R protein expression (Meneses, 1999, 2002). Such regulatory mechanisms may be an important characteristic in the contribution of these receptors in the effects of repeated cocaine exposure, as changes in the influence of the 5-HT_{2A}R and/or 5-HT_{2C}R would alter the normal balance of control of DA mesocorticoaccumbens pathways. Mechanisms of receptor regulation and responsiveness are therefore important for both understanding reinstatement of drug seeking and relapse in humans, as well as an important factor to take into account for development of pharmacotherapies.

The ability of 5-HT_{2A}R and 5-HT_{2C}R manipulations to modulate cocaine-primed and cue-evoked reactivity suggests that these receptors may be integral components of the incentive motivation produced by cocaine and/or cocaine-associated cues. Likewise, given the importance of 5-HT in learning (Meil and Schechter, 1997; Roy et al., 1998; Levin et al., 1999; Grabowski et al., 2000, 2004; Smelson et al., 2002, 2004, 2006; Kampman et al., 2003; Sattar and Bhatia, 2003; Sattar et al., 2003; De La Garza et al., 2005; Reid et al., 2005), the ability of 5-HT_{2A}R antagonists or 5-HT_{2C}R agonists to disrupt retention of a conditioned association between cocaine and environmental stimuli is a real possibility. The suppression of reactivity to cocaine-associated cues induced by a 5-HT_{2A}R antagonist or a 5-HT_{2C}R agonist could have important consequences for the maintenance of abstinence, and might suggest the thorough investigation of the 5-HT_{2A}R and 5-HT_{2C}R as a new avenue to therapeutically enhance abstinence in psychostimulant addiction.

Cocaine and 5-HT₂R pharmacotherapy in humans

A limited number of studies have addressed the potential for serotonergic ligands, and in

particular selective 5-HT₂R ligands, to serve as effective pharmacotherapeutic strategies in addiction. By and large, the majority of studies have utilized non-selective 5-HT₂R antagonists that are clinically available for the treatment of psychosis. For example, studies using atypical antipsychotic drugs with a high 5-HT_{2A}R/D₂-like receptor affinity ratio, including risperidone (Risperdal[®]) and olanzapine (Zyprexa[®]), to reduce cocaine use, relapse and/or craving have demonstrated mixed results (Meil and Schechter, 1997; Smelson et al., 2002, 2004, 2006); the most favourable outcomes were observed for reducing relapse and/or craving among individuals comorbid for schizophrenia and cocaine dependence (Melkersson and Dahl, 2004). Unfortunately, these compounds display comparable affinity for multiple populations of 5-HT₂R, non-5-HT₂R (including DA, muscarinic and adrenergic) and transporter sites and are associated with serious side-effect profiles (Kampman et al., 2003), resulting in poor retention of individuals treated with these compounds in clinical trials (Batki et al., 1993; Covi et al., 1995; Grabowski et al., 1995; Ciraulo et al., 2005; Winhusen et al., 2005).

Others have employed selective serotonin reuptake inhibitors (SSRIs) in outpatient clinical trials for efficacy in cocaine-dependent subjects. Fluoxetine (Prozac[®]), paroxetine (Paxil[®]) and sertraline (Zoloft[®]) were largely ineffective as treatment in outpatient cocaine-dependent subjects as measured by outcomes of treatment retention, cocaine-positive urines or reductions in craving (Moeller et al., 2007). Conversely, another SSRI, citalopram (Celexa[®]), was recently shown to significantly reduce craving and cocaine-positive urines in outpatient studies of cocaine-using subjects (Owens et al., 1997). Citalopram, a highly selective SSRI (3400-fold higher affinity for SERT over NET or DAT) (Millan et al., 1999; Dekeyne et al., 2000; Palvimaki et al., 2005), exhibits in vivo effects consistent with prominent actions as a 5-HT_{2C}R agonist which are dose-dependently blocked by selective 5-HT_{2C}R antagonists (Moeller et al., 2007). Thus, the treatment successes observed with citalopram (Jenck et al., 1993; Palvimaki et al., 1996; Ni and Miledi, 1997) in cocaine-dependent subjects may be due to the net effects of citalopram

to mimic selective 5-HT_{2C}R agonists as opposed to fluoxetine, which acts as a potent antagonist at the 5-HT_{2C}R (Chen et al., 2005; Mnie-Filali et al., 2006). Although not yet examined in cocaine-dependent subjects, escitalopram (Lexapro[®]), the active *S*-enantiomer of citalopram, may be even more effective than citalopram in treatment of psychostimulant dependence. Escitalopram has a 30-fold greater affinity for SERT than does citalopram, and in addition to its property as a SSRI, escitalopram displays a pronounced allosteric effect on the SERT, unique among SSRIs (Sanchez, 2006). Interestingly, *R*-citalopram antagonizes many of the effects of escitalopram possibly due to a disruption of the association of escitalopram with the high-affinity binding site (Laakso et al., 1996; Burke, 2002). A low frequency of side effects and clinical evidence supports its superiority as an antidepressant with faster therapeutic efficacy relative to that of citalopram (Naughton et al., 2000; Weiner et al., 2001, 2003; Wood, 2003; Leysen, 2004; Isaac, 2005). In addition, its affinity for α_1 -adrenoceptors and H₁ histamine receptors is lower than that of citalopram, minimizing the side effects typically associated with actions at these sites.

Taken together, these studies provide some credence that, as predicted from pre-clinical studies demonstrating a role for the 5-HT₂R in cocaine-taking and cocaine-seeking behaviour, selective 5-HT_{2A}R antagonists or 5-HT_{2C}R agonists may be successful in reducing craving and cocaine use. However, the mixed results of these clinical trials also emphasize the need for more selective drugs for use in clinical investigations.

5-HT_{2A}R and 5-HT_{2C}R ligands available for medicine and research

The 5-HT_{2A}R and 5-HT_{2C}R are under investigation as potential therapeutic targets for psychiatric conditions, including schizophrenia, depression and anxiety, obsessive compulsive disorders, as well as sleep disorders and obesity. Active initiatives are underway to develop selective 5-HT_{2A}R and 5-HT_{2C}R ligands with the potential utility in treatment of these disorders. A number of recent

Table 2. Possible investigational drugs with affinity/efficacy at 5-HT_{2A}R and 5-HT_{2C}R^a

Drug	Relevant action(s)	Status
<i>Non-selective 5-HT₂R medications available for clinical trials</i>		
Mirtazapine (Remeron [®])	5-HT _{2A/2C} R antagonist; α_2 -adrenergic receptor antagonist	Marketed as antidepressant; Phase II for comorbid cocaine dependence and depression
mCPP	Preferential 5-HT _{2C} R agonist; 5-HT _{1B} R agonist	Investigational drug in clinical trials
<i>Selective 5-HT₂R compounds potentially available for clinical trials</i>		
M100907 (volinanserin)	Selective 5-HT _{2A} R antagonist	Phase III for sleep disorders; completed Phase II for depression
SR 46349B (eplivanserin)	Selective 5-HT _{2A} R antagonist	Phase III for sleep disorders
APD-125	Selective 5-HT _{2A} R antagonist	Phase II for sleep disorders
ACP-103 (pimavanserin)	5-HT _{2A} R inverse agonist	Phase III for antipsychotic-induced side effects, Parkinson's disease
APD-356 (lorcaserin)	Selective 5-HT _{2C} R agonist	Phase III for obesity
WAY163,909	Selective 5-HT _{2C} R agonist	Pre-clinical
WAY 470	Selective 5-HT _{2C} R agonist	Pre-clinical
LY 448100	Selective 5-HT _{2C} R agonist	Pre-clinical

^asee text for references.

reviews provide detailed descriptions of the variety of 5-HT₂R compounds currently available for pre-clinical and clinical studies (Wikstrom et al., 2002). Therefore, we have chosen to describe in brief a few 5-HT₂R ligands (Table 2) that may show promise for treatment of substance use disorders.

Although progress in understanding 5-HT₂R function in vivo and the clinical potential of 5-HT₂R ligands is escalating as more pharmacologically selective ligands are developed, selective 5-HT_{2A}R antagonists and 5-HT_{2C}R agonists are presently unavailable for use in humans. Among the non-selective 5-HT₂R ligands currently approved for use in humans, mirtazapine and meta-chlorophenylpiperazine (mCPP) display preference for 5-HT₂R. Mirtazapine (Remeron[®]; ORG 3770, also spelled mirtazepine) is a marketed antidepressant drug whose therapeutic effectiveness is linked to its actions as an antagonist at both the 5-HT_{2A}R ($K_i = 2$ nM) and the 5-HT_{2C}R ($K_i = 5.5$ nM) coupled to its potent ability to antagonize central α_2 -adrenergic receptors ($K_i = 58$ nM) (Roth, 2006). While additional actions of mirtazapine at histamine receptors, 5-HT₃R and 5-HT_{1A}R complicate a true

understanding of the mechanisms of action underlying its antidepressant actions relative to its side effects (e.g. sedation, weight gain), mirtazapine, which lacks affinity at DA receptors (Zueco Perez, 2002), provides a distinctive contrast relative to the atypical antipsychotics with potent actions at DA receptors (e.g. risperidone, ritanserin) evaluated to date in treatment studies of cocaine addicts. Indeed, a study conducted in methadone-maintained cocaine-dependent patients suggests that mirtazapine is well tolerated and may improve treatment adherence (Kongsakon et al., 2005). Studies indicate that mirtazapine may be effective at reducing withdrawal symptoms during amphetamine (Liappas et al., 2003, 2004) and alcohol detoxification (Yoon et al., 2006), as well as at reducing craving and increasing mood in patients with alcohol dependence comorbid with depressive disorder (Kleber, 2007). Currently, Phase II clinical trials are underway to examine the effectiveness of mirtazapine to reduce cocaine use in cocaine-dependent patients who also suffer from depression (Hoyer, 1988).

The preferential 5-HT_{2C}R agonist mCPP exhibits modestly higher affinity for the 5-HT_{2C}R

($K_i = 20$ nM) compared to the 5-HT_{2A}R ($K_i = 199$ nM), 5-HT_{1B}R ($K_i = 251$ nM) or 5-HT_{1A}R ($K_i = 316$ nM) (Heisler and Tecott, 2000; Higgins et al., 2001; Dalton et al., 2004; Filip et al., 2004; Frankel and Cunningham, 2004). Despite the lack of selectivity, actions of mCPP mediated through the 5-HT_{2C}R seem to dominate the in vivo behavioural profile elicited by the drug (Winter and Rabin, 1993; Callahan and Cunningham, 1994; Frankel and Cunningham, 2004). In particular, the discriminative stimulus properties of mCPP appear to be primarily mediated via actions at the 5-HT_{2C}R versus other 5-HTRs (Dekeyne et al., 1999), and mCPP has been shown to fully generalize to the discriminative cue of the selective 5-HT_{2C}R agonist RO 60-0175 (Callahan and Cunningham, 1994; Frankel and Cunningham, 2004). mCPP has also been shown to reduce the recognition of the discriminative stimulus effects of cocaine through actions mediated by the 5-HT_{2C}R, but not the 5-HT_{1A}R, 5-HT_{1B}R or 5-HT_{2A}R (Buydens-Branchey et al., 1997). Studies utilizing mCPP have indicated that this compound may be useful in decreasing craving for cocaine in humans (Silverstone and Cowen, 1994). However, the utility of this compound may be limited by the fact that humans exhibit anxiety in response to mCPP exposure (Silverstone and Cowen, 1994), although it is unclear at this time which action(s) of mCPP accounts for this outcome (Rinaldi-Carmona et al., 1992; Kehne et al., 1996).

Several selective 5-HT₂R ligands of interest are in Phase II or III studies with the hope of developing new medications for neurological and/or psychiatric disorders, thereby increasing the possibility that such compounds may soon be available for studies examining their effectiveness in modulating drug use and dependence. The selective 5-HT_{2A}R antagonists M100907 (volinanserin) and SR 46349B (eplivanserin) (Santoni, 2007) are currently in Phase III clinical trials for sleep disorders (Kehne et al., 1996). M100907, which has been utilized in the majority of pre-clinical studies, has 100-fold selectivity for 5-HT_{2A}R ($K_i = 0.85$ nM) versus 5-HT_{2C}R ($K_i = 88$ nM) (Kehne et al., 1996), and at least a 100-fold lower affinity for other receptors, including the 5-HT_{2B}R, α 1-adrenergic receptor and

sigma receptor ($K_i = 261$, 128, and 87 nM, respectively) (McCreary et al., 2003). M100907 selectively blocks behaviours generated by stimulation of 5-HT_{2A}R over a range of doses (NIMH, 2006) and is also being examined in Phase II trials for its efficacy in the treatment of depression (Arena Pharmaceuticals, 2007a). Another 5-HT_{2A}R antagonist, APD-125, was shown to have a favourable safety profile in Phase I clinical trials and Phase II trials for this compound for the maintenance of sleep in insomniacs (Vanover et al., 2006). ACP-103 (pimavanserin), a 5-HT_{2A}R inverse agonist (ACADIA Pharmaceuticals, 2007), recently moved into Phase III clinical trials as a co-therapy to reduce antipsychotic side effects in Parkinson's disease (ACADIA Pharmaceuticals, 2005); Phase II trials revealed that co-therapy with ACP-103 also led to a faster onset of antipsychotic action and an improved side effect profile, including less gain in weight and lower prolactin levels (Arena Pharmaceuticals, 2007b). Thus, several compounds with actions as 5-HT_{2A}R antagonists/inverse agonists are expected to emerge from clinical trials that could be utilized in studies for efficacy in treatment of psychostimulant dependence.

The recent interest in 5-HT_{2C}R agonists in the treatment of psychiatric, neuroendocrine and neurological disorders has resulted in the emergence of several novel 5-HT_{2C}R agonists. APD-356 (lorcaserin), which has ~18-fold selectivity in vitro for the 5-HT_{2C}R versus the 5-HT_{2A}R and >100 fold selectivity over the 5-HT_{2B}R (Thomsen et al., 2008), is currently being examined in Phase III clinical trials for treatment of obesity (Maffiud et al., 2006). Results of Phase II trials revealed that APD-356 was generally well tolerated at all doses assessed to date (Cryan and Lucki, 2000; Welmaker et al., 2000; Dunlop et al., 2005). Several 5-HT_{2C}R agonists are currently being utilized in pre-clinical studies. WAY 163,909 has been shown to be a full 5-HT_{2C}R agonist with no efficacy at 5-HT_{2A}R and a behavioural profile consistent with that of a 5-HT_{2C}R agonist (Marquis et al., 2007). Acute and chronic (21 days) treatment with WAY 163,909 induced a selective decrease in the number of spontaneously active DA neurons in the VTA versus the substantia

nigra, and acute administration induced a selective decrease in DA release in the NAc versus the striatum (Sabb et al., 2004), characteristics that could prove important in treatment of psychostimulant abuse. Other compounds reported to have high selectivity and efficacy as 5-HT_{2C}R agonists include Ly 448100, which is described as a full agonist at 5-HT_{2C}R with 20-fold greater selectivity at the 5-HT_{2C}R over the 5-HT_{2A}R. WAY 470 proved to be a potent ($K_i = 13$ nM) full agonist of the h5-HT_{2C}R in cultured cells; although WAY 470 has not yet been studied in vivo, the chemically similar compound WAY 629 demonstrates a behavioural profile consistent with its actions as a brain-penetrant selective human 5-HT_{2C}R protein agonist. Thus, there are a number of promising selective 5-HT_{2C}R agonists on the horizon that should be available in the near future for studies in humans to examine their utility as treatments of psychostimulant abuse.

Conclusions

The need for more effective medications for the treatment of psychostimulant use and dependence has encouraged researchers to identify novel targets towards which the development of pharmacotherapeutics may be directed. A plethora of pre-clinical data suggest that the 5-HT_{2A}R and 5-HT_{2C}R play important roles in modulating the in vivo effects of cocaine and other drugs of abuse, potentially via modulation of DA neurotransmission within the limbic-cortico-striatal circuitry. In particular, cocaine self-administration studies in rodents indicate that *antagonists* for the 5-HT_{2A}R are effective at reducing relapse to cocaine seeking, while 5-HT_{2C}R *agonists* are effective at reducing both the rate of cocaine intake as well as relapse to cocaine seeking. These studies indicate that 5-HT_{2A}R antagonists and/or 5-HT_{2C}R agonists may be effective at reducing craving and enhancing abstinence in treatment-seeking cocaine addicts, while 5-HT_{2C}R agonists may also be effective for reducing cocaine use in individuals actively using cocaine. Although several selective 5-HT_{2A}R and 5-HT_{2C}R ligands are currently being

developed for, or tested in clinical trials, no selective ligands are presently available for use in clinical trials in cocaine abusers. As such, less selective ligands such as escitalopram, or those with preferential actions as a 5-HT_{2A}R antagonist (e.g. mirtazapine) or a 5-HT_{2C}R agonist (e.g. mCPP), may be useful in the interim for examining proof of concept in the clinical setting.

Abbreviations

5-HT	5-hydroxytryptamine, serotonin
5-HTR	5-HT receptor
CPP	conditioned place preference
DA	dopamine
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
GABA	γ -aminobutyric acid
IF	interfascicular nucleus
mCPP	meta-chlorophenylpiperazine
MDMA	3,4-methylenedioxymethamphetamine
NAc	nucleus accumbens
PBP	parabrachial pigmented nucleus
PCP	phencyclidine
PCPA	<i>p</i> -chlorophenylalanine
PLA ₂	phospholipase A ₂
PLC	phospholipase C
PN	paranigral nucleus
SERT	serotonin reuptake transporter
SNPs	single nucleotide polymorphisms
SSRIs	selective serotonin reuptake inhibitors
VTA	ventral tegmental area

Acknowledgements

We gratefully acknowledge the support from the National Institute on Drug Abuse: DA 00260, DA 06511, DA 07287, DA 13595, DA 15259, DA 020087 and DA 024157.

References

- ACADIA Pharmaceuticals (2005). ACADIA Pharmaceuticals Reports Results from Phase II Study of ACP-103 in

- Schizophrenia Patients With Haloperidol-Induced Akathisia. ACADIA Pharmaceuticals, Inc. Available at: <http://www.pnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/story/12-01-2005/0004225880&EDATE>. Accessed February 4, 2008.
- ACADIA Pharmaceuticals (2007). ACADIA Pharmaceuticals Initiates Phase III Trial with Pimavanserin in Patients with Parkinson's Disease Psychosis. ACADIA Pharmaceuticals, Inc. Available at: <http://news.acadia-pharm.com/phoenix.zhtml?c=125180&p=irol-newsArticle&ID=1013660&highlight=ACP-103>. Accessed February 4, 2008.
- Ait-Daoud, N., Malcolm, R.J., Jr. and Johnson, B.A. (2006) An overview of medications for the treatment of alcohol withdrawal and alcohol dependence with an emphasis on the use of older and newer anticonvulsants. *Addict. Behav.*, 31(9): 1628–1649.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113(2): 296–320.
- Allan, A., Galindo, R., Chynoweth, J., Engel, S. and Savage, D. (2001) Conditioned place preference for cocaine is attenuated in mice over-expressing the 5-HT(3) receptor. *Psychopharmacology*, 158(1): 18–27.
- Arena Pharmaceuticals (2007a). Arena Pharmaceuticals Announces Positive Phase 2 Clinical Trial Results of APD125 for the Treatment of Insomnia. Arena Pharmaceuticals, Inc. Available at: <http://phx.corporate-ir.net/phoenix.zhtml?c=121703&p=irol-newsArticle&ID=1055090&highlight>. Accessed February 4, 2008.
- Arena Pharmaceuticals (2007b). Arena Pharmaceuticals Initiates Second and Third Pivotal Trials Evaluating Lorcaserin for the Treatment of Obesity. Arena Pharmaceuticals, Inc. Available at: <http://phx.corporate-ir.net/phoenix.zhtml?c=121703&p=irol-newsArticle&ID=1087081&highlight>. Accessed February 4, 2008.
- Auclair, A., Blanc, G., Glowinski, J. and Tassin, J.P. (2004a) Role of serotonin 2A receptors in the D-amphetamine-induced release of dopamine: comparison with previous data on alpha1b-adrenergic receptors. *J. Neurochem.*, 91(2): 318–326.
- Auclair, A., Drouin, C., Cotecchia, S., Glowinski, J. and Tassin, J.P. (2004b) 5-HT_{2A} and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. *Eur. J. Neurosci.*, 20(11): 3073–3084.
- Bankson, M.G. and Cunningham, K.A. (2001) 3,4-Methylenedioxymethamphetamine (MDMA) as a unique model of serotonin receptor function and serotonin-dopamine interactions. *J. Pharmacol. Exp. Ther.*, 297(3): 846–852.
- Bankson, M.G. and Cunningham, K.A. (2002) Pharmacological studies of the acute effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT(1B/1D) and 5-HT(2) receptors. *Neuropsychopharmacology*, 26(1): 40–52.
- Bardo, M.T. and Bevins, R.A. (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, 153(1): 31–43.
- Batki, S.L., Manfredi, L.B., Jacob, P., III and Jones, R.T. (1993) Fluoxetine for cocaine dependence in methadone maintenance: quantitative plasma and urine cocaine/benzoylgonine concentrations. *J. Clin. Psychopharmacol.*, 13: 243–250.
- Battaglia, G., Cabrera-Vera, T.M. and Van De Kar, L.D. (2000) Prenatal cocaine exposure potentiates 5-HT(2a) receptor function in male and female rat offspring. *Synapse*, 35(3): 163–172.
- Baumann, M.H. and Rothman, R.B. (1998) Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. *Biol. Psychiatry*, 44(7): 578–591.
- Berg, K.A., Harvey, J.A., Spampinato, U. and Clarke, W.P. (2005) Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. *Trends Pharmacol. Sci.*, 26(12): 625–630.
- Berg, K.A., Maayani, S., Goldfarb, J., Scaramellini, C., Leff, P. and Clarke, W.P. (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.*, 54(1): 94–104.
- Berg, K.A., Stout, B.D., Cropper, J.D., Maayani, S. and Clarke, W.P. (1999) Novel actions of inverse agonists on 5-HT_{2C} receptor systems. *Mol. Pharmacol.*, 55(5): 863–872.
- Berg, K.A., Stout, B.D., Maayani, S. and Clarke, W.P. (2001) Differences in rapid desensitization of 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptor-mediated phospholipase C activation. *J. Pharmacol. Exp. Ther.*, 299(2): 593–602.
- Bjork, J.M., Moeller, F.G., Dougherty, D.M., Swann, A.C., Machado, M.A. and Hanis, C.L. (2002) Serotonin 2a receptor T102C polymorphism and impaired impulse control. *Am. J. Med. Genet.*, 114(3): 336–339.
- Boess, F.G. and Martin, I.L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology*, 33: 275–317.
- Bonaccorso, S., Meltzer, H.Y., Li, Z., Dai, J., Alboszta, A.R. and Ichikawa, J. (2002) SR46349-B, a 5-HT(2A/2C) receptor antagonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Neuropsychopharmacology*, 27(3): 430–441.
- Bonhaus, D.W., Bach, C., DeSouza, A., Salazar, F.H.R., Matsuoka, B.D., Zuppan, P., Chan, H.W. and Eglen, R.M. (1995) The pharmacology and distribution of human 5-hydroxytryptamine_{2B} (5-HT_{2B}) receptor gene products: comparison with 5-HT_{2A} and 5-HT_{2C} receptors. *Br. J. Pharmacol.*, 115: 622–628.
- Bortolozzi, A., az-Mataix, L., Scorza, M.C., Celada, P. and Artigas, F. (2005) The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. *J. Neurochem.*, 95(6): 1597–1607.
- Brady, K.T., Myrick, H. and McElroy, S. (1998) The relationship between substance use disorders, impulse control disorders, and pathological aggression. *Am. J. Addict.*, 7(3): 221–230.
- Broderick, P.A., Olabisi, O.A., Rahni, D.N. and Zhou, Y. (2004) Cocaine acts on accumbens monoamines and locomotor behavior via a 5-HT_{2A/2C} receptor mechanism as

- shown by ketanserin: 24-h follow-up studies. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28(3): 547–557.
- Brown, C.M., Fletcher, P.J. and Coscina, D.V. (1998) Acute amino acid loads that deplete brain serotonin fail to alter behavior. *Pharmacol. Biochem. Behav.*, 59(1): 115–121.
- Bubar, M.J. and Cunningham, K.A. (2006) Serotonin 5-HT_{2A} and 5-HT_{2C} receptors as potential targets for modulation of psychostimulant use and dependence. *Curr. Top. Med. Chem.*, 6: 1971–1985.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Bubar, M.J., Seitz, P.K., Thomas, M.L. and Cunningham, K.A. (2005) Validation of a selective serotonin 5-HT_{2C} receptor antibody for utilization in fluorescence immunohistochemistry studies. *Brain Res.*, 1063(2): 105–113.
- Burbassi, S. and Cervo, L. (2008) Stimulation of serotonin(2C) receptors influences cocaine-seeking behavior in response to drug-associated stimuli in rats. *Psychopharmacology (Berl.)*, 196(1): 15–27.
- Burke, W.J. (2002) Escitalopram. *Expert Opin. Investig. Drugs*, 11(10): 1477–1486.
- Burmeister, J.J., Lungren, E.M., Kirschner, K.F. and Neisewander, J.L. (2004) Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*, 29(4): 660–668.
- Burnet, P.W.J., Eastwood, S.L., Lacey, K. and Harrison, P.J. (1995) The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res.*, 676: 157–168.
- Buydens-Branchey, L., Branchey, M., Ferguson, P., Hudson, J. and McKernin, C. (1997) The meta-chlorophenylpiperazine challenge test in cocaine addicts: hormonal and psychological responses. *Biol. Psychiat.*, 41(11): 1071–1086.
- Callahan, P.M. and Cunningham, K.A. (1994) Involvement of 5-HT_{2C} receptors in mediating the discriminative stimulus properties of *m*-chlorophenylpiperazine (MCP). *Eur. J. Pharmacol.*, 257: 27–38.
- Callahan, P.M. and Cunningham, K.A. (1995) Modulation of the discriminative stimulus properties of cocaine by 5-HT_{1B} and 5-HT_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 274: 1414–1424.
- Callahan, P.M. and Cunningham, K.A. (1997) Comparison of fluoxetine with 5-HT_{1A} and 5-HT_{1B} receptor agonists to modulate the discriminative stimulus properties of cocaine in rats. *Neuropharmacology*, 36: 373–381.
- Callahan, P.M., De La Garza, R. and Cunningham, K.A. (1997) Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. *Pharmacol. Biochem. Behav.*, 57(3): 601–607.
- Carrasco, G.A. and Battaglia, G. (2007) Withdrawal from a single exposure to cocaine increases 5-HT_{2A} receptor and G protein function. *Neuroreport*, 18(1): 51–55.
- Carrasco, G.A., Damjanoska, K.J., D'Souza, D.N., Zhang, Y., Garcia, F., Battaglia, G., Muma, N.A. and Van De Kar, L.D. (2004) Short-term cocaine treatment causes neuroadaptive changes in Galphaq and Galpha11 proteins in rats undergoing withdrawal. *J. Pharmacol. Exp. Ther.*, 311(1): 349–355.
- Carrasco, G.A., Zhang, Y., Damjanoska, K.J., D'Souza, D.N., Garcia, F., Battaglia, G., Muma, N.A. and Van De Kar, L.D. (2003) A region-specific increase in Galphaq and Galpha11 proteins in brains of rats during cocaine withdrawal. *J. Pharmacol. Exp. Ther.*, 307(3): 1012–1019.
- Chen, F., Larsen, M.B., Sanchez, C. and Wiborg, O. (2005) The *S*-enantiomer of *R,S*-citalopram, increases inhibitor binding to the human serotonin transporter by an allosteric mechanism. Comparison with other serotonin transporter inhibitors. *Eur. Neuropsychopharmacol.*, 15(2): 193–198.
- Ciraulo, D.A., Sarid-Segal, O., Knapp, C.M., Ciraulo, A.M., LoCastro, J., Bloch, D.A., Montgomery, M.A., Leiderman, D.B. and Elkashef, A. (2005) Efficacy screening trials of paroxetine, pentoxifylline, riluzole, pramipexole and venlafaxine in cocaine dependence. *Addiction*, 100(Suppl. 1): 12–22.
- Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P. and Fone, K.C. (2000) Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. *Neuropharmacology*, 39(1): 123–132.
- Conde, F., Audinat, E., Maire-Lepoivre, E. and Crepel, F. (1990) Afferent connections of the medial frontal cortex of the rat: a study using retrograde transport of fluorescent dyes. I. Thalamic afferents. *Brain Res. Bull.*, 24: 341–354.
- Cornea-Hebert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, 409(2): 187–209.
- Covi, L., Hess, J.M., Kreiter, N.A. and Haertzen, C.A. (1995) Effects of combined fluoxetine and counseling in the outpatient treatment of cocaine abusers. *Am. J. Drug Alcohol Abuse*, 21: 327–344.
- Cryan, J.F. and Lucki, I. (2000) Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine(2C) receptors. *J. Pharmacol. Exp. Ther.*, 295(3): 1120–1126.
- Cunningham, K.A. and Callahan, P.M. (1994) Neurobehavioral pharmacology of cocaine: role for serotonin in its locomotor and discriminative stimulus effects. In: Erinoff L. and Brown R.M. (Eds.), *Neurobiological Models for Evaluating Mechanisms Underlying Cocaine Addiction*. US Government Printing Office, pp. 40–66.
- Dalton, G.L., Lee, M.D., Kennett, G.A., Dourish, C.T. and Clifton, P.G. (2004) mCPP-induced hyperactivity in 5-HT_{2C} receptor mutant mice is mediated by activation of multiple 5-HT receptor subtypes. *Neuropharmacology*, 46(5): 663–671.
- Darmani, N.A. and Ahmad, B. (2000) Early postnatal cocaine exposure causes sequential, dose-dependent, enduring but reversible supersensitivity in 5-HT_{2A} receptor-mediated function during development in male mice. *Neurotoxicol. Teratol.*, 22(1): 61–69.
- Darmani, N.A., Martin, B.R. and Glennon, R.A. (1990) Withdrawal from chronic treatment with (±)-DOI causes super-sensitivity to 5-HT₂ receptor-induced head-twitch behaviour in mice. *Eur. J. Pharmacol.*, 186: 115–118.

- De Deurwaerdere, P., Navailles, S., Berg, K.A., Clarke, W.P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24(13): 3235–3241.
- De Deurwaerdere, P. and Spampinato, U. (1999) Role of serotonin(2A) and serotonin(2B/2C) receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73(3): 1033–1042.
- Dekeyne, A., Denorme, B., Monneyron, S. and Millan, M.J. (2000) Citalopram reduces social interaction in rats by activation of serotonin (5-HT)_{2C} receptors. *Neuropharmacology*, 39(6): 1114–1117.
- Dekeyne, A., Girardon, S. and Millan, M.J. (1999) Discriminative stimulus properties of the novel serotonin (5-HT)_{2C} receptor agonist, RO 60-0175: a pharmacological analysis. *Neuropharmacology*, 38(3): 415–423.
- De La Garza, R., Callahan, P.M. and Cunningham, K.A. (1996) Detailed investigations of 5-HT₃ compounds in a drug discrimination model. *Pharmacol. Biochem. Behav.*, 54: 533–540.
- De La Garza, R., Callahan, P.M. and Cunningham, K.A. (1998) The discriminative stimulus effects of cocaine: effects of microinfusion of cocaine, a 5-HT_{1A} agonist or antagonist into the ventral tegmental area. *Psychopharmacology*, 137: 71–76.
- De La Garza, R. and Cunningham, K.A. (2000) The effects of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin on spontaneous activity and cocaine-induced hyperactivity and behavioral sensitization. *J. Pharmacol. Exp. Ther.*, 292: 610–617.
- De La Garza, R., Newton, T.F. and Kalechstein, A.D. (2005) Risperidone diminishes cocaine-induced craving. *Psychopharmacology*, 178(2–3): 347–350.
- de Wit, H. and Stewart, J. (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl.)*, 75(2): 134–143.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91(2): 587–597.
- Di Giovanni, G., Di, M.V., Di Mascio, M. and Esposito, E. (2000) Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. *Synapse*, 35(1): 53–61.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38(8): 1195–1205.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. *Brain Res.*, 865(1): 85–90.
- Doherty, M.D. and Pickel, V.M. (1999) Ultrastructural localization of the 5-HT_{2A} receptor in dopaminergic neurons in the ventral tegmental area. *Soc. Neurosci. Abstr.*, 25: p. 1202.
- Donna, M.P., James, K.R. and Roger, D.S. (2002) Behavioral effects of cocaine and dopaminergic strategies for preclinical medication development. *Psychopharmacology*, 163(3–4): 265–282.
- Dunlop, J., Sabb, A.L., Mazandarani, H., Zhang, J., Kalgaonker, S., Shukhina, E., Sukoff, S., Vogel, R.L., Stack, G., Schechter, L., Harrison, B.L. and Rosenzweig-Lipson, S. (2005) WAY-163909 ((7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole); a novel 5-HT_{2C} receptor selective agonist with anorectic activity. *J. Pharmacol. Exp. Ther.*, 313(2): 862–869.
- Dworkin, S.I., Co, C. and Smith, J.E. (1995) Rat brain neurotransmitter turnover rates altered during withdrawal from chronic cocaine administration. *Brain Res.*, 682: 116–126.
- Eberle-Wang, K., Mikeldadze, Z., Uryu, K. and Chesselet, M.F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384(2): 233–247.
- El-Mallakh, R.S. and Abraham, H.D. (2007) MDMA (ecstasy). *Ann. Clin. Psychiatry*, 19(1): 45–52.
- Ettenberg, A. (1989) Dopamine, neuroleptics and reinforced behavior. *Neurosci. Biobehav. Rev.*, 13(2–3): 105–111.
- Everitt, B.J. and Wolf, M.E. (2002) Psychomotor stimulant addiction: a neural systems perspective. *J. Neurosci.*, 22(9): 3312–3320.
- Fantegrossi, W.E., Ullrich, T., Rice, K.C., Woods, J.H. and Winger, G. (2002) 3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) and its stereoisomers as reinforcers in rhesus monkeys: serotonergic involvement. *Psychopharmacology (Berl.)*, 161(4): 356–364.
- Felder, C.C., Kanterman, R.Y., Ma, A.L. and Axelrod, J. (1990) Serotonin stimulates phospholipase A2 and the release of arachidonic acid in hippocampal neurons by a type 2 serotonin receptor that is independent of inositolphospholipid hydrolysis. *Proc. Natl. Acad. Sci. USA*, 87(6): 2187–2191.
- Filip, M., Bubar, M.J. and Cunningham, K.A. (2004) Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT₂ receptor subtypes to the hyperlocomotor effects of cocaine: acute and chronic pharmacological analyses. *J. Pharmacol. Exp. Ther.*, 310(3): 1246–1254.
- Filip, M., Bubar, M.J. and Cunningham, K.A. (2006) Contribution of serotonin (5-HT) 5-HT₂ receptor subtypes to the discriminative stimulus effects of cocaine in rats. *Psychopharmacology*, 183(4): 482–489.
- Filip, M. and Cunningham, K.A. (2002) Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacol. Biochem. Behav.*, 71(4): 745–756.
- Filip, M. and Cunningham, K.A. (2003) Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin(2C) (5-HT(2C)) receptors in rat prefrontal cortex. *J. Pharmacol. Exp. Ther.*, 306(2): 734–743.
- Fink, K.B. and Gothert, M. (2007) 5-HT receptor regulation of neurotransmitter release. *Pharmacol. Rev.*, 59(4): 360–417.

- Fletcher, P.J., Chintoh, A.F., Sinyard, J. and Higgins, G.A. (2004) Injection of the 5-HT_{2C} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. *Neuropsychopharmacology*, 29(2): 308–318.
- Fletcher, P.J., Grottick, A.J. and Higgins, G.A. (2002a) Differential effects of the 5-HT_{2A} receptor antagonist M100,907 and the 5-HT_{2C} receptor antagonist SB242,084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27(4): 576–586.
- Fletcher, P.J., Korth, K.M., Robinson, S.R. and Baker, G.B. (2002b) Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement. *Psychopharmacology (Berl.)*, 162(3): 282–291.
- Fletcher, P.J., Rizos, Z., Sinyard, J., Tampakeras, M. and Higgins, G.A. (2008) The 5-HT(2C) receptor agonist Ro60-0175 reduces cocaine self-administration and reinstatement induced by the stressor yohimbine, and contextual cues. *Neuropsychopharmacology*, 33(6): 1402–1412.
- Fletcher, P.J., Sinyard, J. and Higgins, G.A. (2006) The effects of the 5-HT(2C) receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists. *Psychopharmacology (Berl.)*, 187(4): 515–525.
- Frankel, P.S. and Cunningham, K.A. (2004) m-Chlorophenylpiperazine (mCPP) modulates the discriminative stimulus effects of cocaine through actions at the 5-HT_{2C} receptor. *Behav. Neurosci.*, 118(1): 157–162.
- Gonzalez-Burgos, G., Krimer, L.S., Povysheva, N.V., Barriovenue, G. and Lewis, D.A. (2005) Functional properties of fast spiking interneurons and their synaptic connections with pyramidal cells in primate dorsolateral prefrontal cortex. *J. Neurophysiol.*, 93(2): 942–953.
- Grabowski, J., Rhoades, H., Elk, R., Schmitz, J., Davis, C., Creson, D. and Kirby, K. (1995) Fluoxetine is ineffective for treatment of cocaine dependence or concurrent opiate and cocaine dependence: two placebo-controlled, double-blind trials. *J. Clin. Psychopharmacol.*, 15: 163–174.
- Grabowski, J., Rhoades, H., Silverman, P., Schmitz, J.M., Stotts, A., Creson, D. and Bailey, R. (2000) Risperidone for the treatment of cocaine dependence: randomized, double-blind trial. *J. Clin. Psychopharmacol.*, 20(3): 305–310.
- Grabowski, J., Shearer, J., Merrill, J. and Negus, S.S. (2004) Agonist-like replacement pharmacotherapy for stimulant abuse and dependence. *Addict. Behav.*, 29(7): 1439–1464.
- Gray, J.A., Compton-Toth, B.A. and Roth, B.L. (2003) Identification of two serine residues essential for agonist-induced 5-HT_{2A} receptor desensitization. *Biochemistry*, 42(36): 10853–10862.
- Gray, J.A. and Roth, B.L. (2001) Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res. Bull.*, 56(5): 441–451.
- Green, A.R. (2006) Neuropharmacology of 5-hydroxytryptamine. *Br. J. Pharmacol.*, 147(Suppl 1): S145–S152.
- Grottick, A.J., Corrigan, W.A. and Higgins, G.A. (2001) Activation of 5-HT_{2C} receptors reduces the locomotor and rewarding effects of nicotine. *Psychopharmacology (Berl.)*, 157(3): 292–298.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT(2C) receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295(3): 1183–1191.
- Gurevich, I., Englander, M.T., Adlersberg, M., Siegal, N.B. and Schmauss, C. (2002) Modulation of serotonin 2C receptor editing by sustained changes in serotonergic neurotransmission. *J. Neurosci.*, 22(24): 10529–10532.
- Halliday, G.M. and Tork, I. (1989) Serotonin-like immunoreactive cells and fibres in the rat ventromedial mesencephalic tegmentum. *Brain Res. Bull.*, 22: 725–735.
- Harris, G.C., Altomare, K. and Aston-Jones, G. (2001) Preference for a cocaine-associated environment is attenuated by augmented accumbal serotonin in cocaine withdrawn rats. *Psychopharmacology (Berl.)*, 156(1): 14–22.
- Heisler, L.K. and Tecott, L.H. (2000) A paradoxical locomotor response in serotonin 5-HT(2C) receptor mutant mice. *J. Neurosci.*, 20(8): p. RC71.
- Herrick-Davis, K., Grinde, E., Harrigan, T.J. and Mazurkiewicz, J.E. (2005) Inhibition of serotonin 5-hydroxytryptamine_{2C} receptor function through heterodimerization: receptor dimers bind two molecules of ligand and one G-protein. *J. Biol. Chem.*, 280(48): 40144–40151.
- Herrick-Davis, K., Grinde, E. and Mazurkiewicz, J.E. (2004) Biochemical and biophysical characterization of serotonin 5-HT_{2C} receptor homodimers on the plasma membrane of living cells. *Biochemistry*, 43(44): 13963–13971.
- Herrick-Davis, K., Grinde, E. and Niswender, C.M. (1999) Serotonin 5-HT_{2C} receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. *J. Neurochem.*, 73(4): 1711–1717.
- Herrick-Davis, K., Weaver, B.A., Grinde, E. and Mazurkiewicz, J.E. (2006) Serotonin 5-HT_{2C} receptor homodimer biogenesis in the endoplasmic reticulum: real-time visualization with confocal fluorescence resonance energy transfer. *J. Biol. Chem.*, 281(37): 27109–27116.
- Higgins, G.A. and Fletcher, P.J. (2003) Serotonin and drug reward: focus on 5-HT(2C) receptors. *Eur. J. Pharmacol.*, 480(1–3): 151–162.
- Higgins, G.A., Ouagazzal, A.M. and Grottick, A.J. (2001) Influence of the 5-HT(2C) receptor antagonist SB242,084 on behaviour produced by the 5-HT(2) agonist Ro60-0175 and the indirect 5-HT agonist dexfenfluramine. *Br. J. Pharmacol.*, 133(4): 459–466.
- Hoyer, D. (1988) Functional correlates of serotonin 5-HT₁ recognition sites. *J. Recept. Res.*, 8: 59–81.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Hughes, J.R., Stead, L.F. and Lancaster, T. (2007) Antidepressants for smoking cessation. *Cochrane Database Syst. Rev.*, (1): p. CD000031.

- Hyman, S.E., Malenka, R.C. and Nestler, E.J. (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.*, 29: 565–598.
- Ikemoto, K., Nishimura, A., Okado, N., Mikuni, M., Nishi, K. and Nagatsu, I. (2000) Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. *Brain Res.*, 853(2): 377–380.
- Isaac, M. (2005) Serotonergic 5-HT_{2C} receptors as a potential therapeutic target for the design antiepileptic drugs. *Curr. Top. Med. Chem.*, 5: 59–67.
- Iwamoto, K. and Kato, T. (2002) Effects of cocaine and reserpine administration on RNA editing of rat 5-HT_{2C} receptor estimated by primer extension combined with denaturing high-performance liquid chromatography. *Pharmacogenomics J.*, 2(5): 335–340.
- Jakab, R.L. and Goldman-Rakic, P.S. (2000) Segregation of serotonin 5-HT_{2A} and 5-HT₃ receptors in inhibitory circuits of the primate cerebral cortex. *J. Comp. Neurol.*, 417(3): 337–348.
- Jenck, F., Moreau, J.-L., Mutel, V., Martin, J.R. and Haefely, W.E. (1993) Evidence for a role of 5-HT_{1C} receptors in the antiserotonergic properties of some antidepressant drugs. *Eur. J. Pharmacol.*, 231: 223–229.
- Jones, B.J. and Blackburn, T.P. (2002) The medical benefit of 5-HT research. *Pharmacol. Biochem. Behav.*, 71(4): 555–568.
- Kalivas, P.W. and McFarland, K. (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology (Berl.)*, 168(1–2): 44–56.
- Kalivas, P.W. and Volkow, N.D. (2005) The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry*, 162(8): 1403–1413.
- Kampman, K.M., Pettinati, H., Lynch, K.G., Sparkman, T. and O'Brien, C.P. (2003) A pilot trial of olanzapine for the treatment of cocaine dependence. *Drug Alcohol Depend.*, 70(3): 265–273.
- Katsuki, H. and Okuda, S. (1995) Arachidonic acid as a neurotoxic and neurotrophic substance. *Prog. Neurobiol.*, 46(6): 607–636.
- Katz, J.L. and Higgins, S.T. (2003) The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology (Berl.)*, 168(1–2): 21–30.
- Kauer, J.A. and Malenka, R.C. (2007) Synaptic plasticity and addiction. *Nat. Rev. Neurosci.*, 8(11): 844–858.
- Kaumann, A.J. and Levy, F.O. (2006) 5-Hydroxytryptamine receptors in the human cardiovascular system. *Pharmacol. Ther.*, 111(3): 674–706.
- Kehne, J.H., Baron, B.M., Carr, A.A., Chaney, S.F., Elands, J., Feldman, D.J., Frank, R.A., van Giersbergen, P.L., McCloskey, T.C., Johnson, M.P., Mccarty, D.R., Poirot, M., Senyah, Y., Siegel, B.W. and Widmaier, C. (1996) Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. *J. Pharmacol. Exp. Ther.*, 277(2): 968–981.
- Kelley, A.E. and Berridge, K.C. (2002) The neuroscience of natural rewards: relevance to addictive drugs. *J. Neurosci.*, 22(9): 3306–3311.
- Kleber, H. (2007) A Free Treatment Study for Cocaine Dependence Looking at the Effectiveness of Mirtazapine in Treating Cocaine Dependent Individuals Who Also Suffer From Depression. *Clinicaltrials.gov*. Available at: <http://clinicaltrials.gov/ct2/show/NCT00249444?term=mirtazapine&rank=4>. Accessed 2008.
- Koe, B.K. (1976) Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J. Pharmacol. Exp. Ther.*, 199: 649–661.
- Kongsakon, R., Papadopoulos, K.I. and Saguansiritham, R. (2005) Mirtazapine in amphetamine detoxification: a placebo-controlled pilot study. *Int. Clin. Psychopharmacol.*, 20(5): 253–256.
- Koob, G.F. and Le, M.M. (2001) Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*, 24(2): 97–129.
- Kreek, M.J., Bart, G., Lilly, C., LaForge, K.S. and Nielsen, D.A. (2005) Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. *Pharmacol. Rev.*, 57(1): 1–26.
- Kursar, J.D., Nelson, D.L., Wainscott, D.B. and Baez, M. (1994) Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine_{2B} receptor. *Mol. Pharmacol.*, 46: 227–234.
- Laakso, A., Palvimäki, E.-P., Kuoppamäki, M., Syvälahti, E. and Hietala, J. (1996) Chronic citalopram and fluoxetine treatments upregulate 5-HT_{2C} receptors in the rat choroid plexus. *Neuropsychopharmacology*, 15(2): 143–151.
- Levin, E.D. and Rezvani, A.H. (2007) Nicotinic interactions with antipsychotic drugs, models of schizophrenia and impacts on cognitive function. *Biochem. Pharmacol.*, 74(8): 1182–1191.
- Levin, F.R., McDowell, D., Evans, S.M., Brooks, D., Spano, C. and Nunes, E.V. (1999) Pergolide mesylate for cocaine abuse: a controlled preliminary trial. *Am. J. Addict.*, 8(2): 120–127.
- Leysen, J.E. (2004) 5-HT₂ receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3(1): 11–26.
- Liappas, J., Paparrigopoulos, T., Malitas, P., Tzavellas, E. and Christodoulou, G. (2004) Mirtazapine improves alcohol detoxification. *J. Psychopharmacol.*, 18(1): 88–93.
- Liappas, J., Paparrigopoulos, T., Tzavellas, E. and Christodoulou, G. (2003) Alcohol detoxification and social anxiety symptoms: a preliminary study of the impact of mirtazapine administration. *J. Affect. Disord.*, 76(1–3): 279–284.
- Liu, S., Bubar, M.J., Lanfranco, M.F., Hillman, G.R. and Cunningham, K.A. (2007) Serotonin(2C) receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*, 146: 1667–1688.
- Liu, S. and Cunningham, K.A. (2006) Serotonin(2C) receptors (5-HT(2C)R) control expression of cocaine-induced conditioned hyperactivity. *Drug Alcohol Depend.*, 81(3): 275–282.
- Lopez-Gimenez, J.F., Mengod, G., Palacios, J.M. and Vilario, M.T. (2001a) Regional distribution and cellular localization

- of 5-HT_{2C} receptor mRNA in monkey brain: comparison with [3 H]mesulergine binding sites and choline acetyltransferase mRNA. *Synapse*, 42(1): 12–26.
- Lopez-Gimenez, J.F., Vilaro, M.T., Palacios, J.M. and Mengod, G. (2001b) Mapping of 5-HT_{2A} receptors and their mRNA in monkey brain: [3 H]MDL100,907 autoradiography and in situ hybridization studies. *J. Comp. Neurol.*, 429(4): 571–589.
- Lu, L., Shepard, J.D., Scott, H.F. and Shaham, Y. (2003) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. *Neurosci. Biobehav. Rev.*, 27(5): 457–491.
- Luttgen, M., Ove, O.S. and Meister, B. (2004) Chemical identity of 5-HT_{2A} receptor immunoreactive neurons of the rat septal complex and dorsal hippocampus. *Brain Res.*, 1010(1–2): 156–165.
- Maffuid, P., Smith, B., Thomsen, B., Behan, D., Lu, X.X., Agarwal, R., Prosser, W., Anderson, C., Grilley, D., Donahue, D., Sleet, R. and Shanahan, W. (2006) 5-HT_{2C} Agonists in Obesity Drug Discovery and Development: Success and Challenges. Arena Pharmaceuticals. Available at: <http://www.sabpa.org/web/bio-pharma06/PaulMaffuid-ArenaLorcaserinSDBiopharmaConference2006061006%5B2%5D.pdf>. Accessed February 4, 2008.
- Malhotra, A.K., Murphy, G.M., Jr. and Kennedy, J.L. (2004) Pharmacogenetics of psychotropic drug response. *Am. J. Psychiatry*, 161(5): 780–796.
- Marion, S., Weiner, D.M. and Caron, M.G. (2004) RNA editing induces variation in desensitization and trafficking of 5-hydroxytryptamine 2c receptor isoforms. *J. Biol. Chem.*, 279(4): 2945–2954.
- Marona-Lewicka, D. and Nichols, D.E. (1997) 5-HT_{2A/2C} receptor agonists potentiate the discriminative cue of (+)-amphetamine in the rat. *Neuropharmacology*, 36(10): 1471–1475.
- Marquis, K.L., Sabb, A.L., Logue, S.F., Brennan, J.A., Piesla, M.J., Comery, T.A., Grauer, S.M., Ashby, C.R., Jr., Nguyen, H.Q., Dawson, L.A., Barrett, J.E., Stack, G., Meltzer, H.Y., Harrison, B.L. and Rosenzweig-Lipson, S. (2007) WAY-163909 [(7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole]: a novel 5-hydroxytryptamine 2C receptor-selective agonist with preclinical antipsychotic-like activity. *J. Pharmacol. Exp. Ther.*, 320(1): 486–496.
- Martin, G.R. and Humphrey, P.P.A. (1994) Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology*, 33: 261–273.
- Maurel, S., Schreiber, R. and De, V.J. (1998) Role of 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors in the generalization of 5-HT receptor agonists to the ethanol cue in the rat. *Behav. Pharmacol.*, 9(4): 337–343.
- Maurel-Remy, S., Bervoets, K. and Millan, M.J. (1995) Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100,907 in rats reflects antagonism of 5-HT_{2A} receptors. *Eur. J. Pharmacol.*, 280(2): R9–R11.
- Maydanovich, O. and Beal, P.A. (2006) Breaking the central dogma by RNA editing. *Chem. Rev.*, 106(8): 3397–3411.
- McCreary, A.C., Bankson, M.G. and Cunningham, K.A. (1999) Pharmacological studies of the acute and chronic effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine_{1A} and 5-hydroxytryptamine_{1B/1D} receptors. *J. Pharmacol. Exp. Ther.*, 290: 965–973.
- McCreary, A.C. and Cunningham, K.A. (1999) The effects of the 5-HT_{2C/2B} antagonist SB 206553 on cocaine-induced locomotor activity. *Neuropsychopharmacology*, 20: 556–564.
- McCreary, A.C., Filip, M. and Cunningham, K.A. (2003) Discriminative stimulus properties of (+/–)-fenfluramine: the role of 5-HT₂ receptor subtypes. *Behav. Neurosci.*, 117(2): 212–221.
- McDonald, A.J. and Mascagni, F. (2007) Neuronal localization of 5-HT type 2A receptor immunoreactivity in the rat basolateral amygdala. *Neuroscience*, 146(1): 306–320.
- McGrew, L., Chang, M.S. and Sanders-Bush, E. (2002) Phospholipase D activation by endogenous 5-hydroxytryptamine 2C receptors is mediated by Galpha13 and pertussis toxin-insensitive Gbetagamma subunits. *Mol. Pharmacol.*, 62(6): 1339–1343.
- McLellan, A.T., Lewis, D.C., O'Brien, C.P. and Kleber, H.D. (2000) Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *JAMA*, 284(13): 1689–1695.
- McLellan, A.T., Woody, G.E., Metzger, D., McKay, J., Durrell, J., Alterman, A.I. and O'Brien, C.P. (1996) Evaluating the effectiveness of addiction treatments: reasonable expectations, appropriate comparisons. *Milbank Q.*, 74(1): 51–85.
- McMahon, L.R. and Cunningham, K.A. (1999) Antagonism of 5-HT₄ receptors attenuates hyperactivity induced by cocaine: putative role for 5-HT₄ receptors in the nucleus accumbens shell. *J. Pharmacol. Exp. Ther.*, 291: 300–307.
- McMahon, L.R. and Cunningham, K.A. (2001a) Antagonism of 5-hydroxytryptamine(2a) receptors attenuates the behavioral effects of cocaine in rats. *J. Pharmacol. Exp. Ther.*, 297(1): 357–363.
- McMahon, L.R. and Cunningham, K.A. (2001b) Role of 5-HT(2a) and 5-HT(2B/2C) receptors in the behavioral interactions between serotonin and catecholamine reuptake inhibitors. *Neuropsychopharmacology*, 24(3): 319–329.
- McMahon, L.R., Filip, M. and Cunningham, K.A. (2001) Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)_{2A} and 5-HT_{2C} receptors. *J. Neurosci.*, 21(19): 7781–7787.
- Meil, W.M. and Schechter, M.D. (1997) Olanzapine attenuates the reinforcing effects of cocaine. *Eur. J. Pharmacol.*, 340(1): 17–26.
- Melkersson, K. and Dahl, M.L. (2004) Adverse metabolic effects associated with atypical antipsychotics: literature review and clinical implications. *Drugs*, 64(7): 701–723.
- Meneses, A. (1999) 5-HT system and cognition. *Neurosci. Biobehav. Rev.*, 23(8): 1111–1125.
- Meneses, A. (2002) Involvement of 5-HT(2A/2B/2C) receptors on memory formation: simple agonism, antagonism, or inverse agonism? *Cell Mol. Neurobiol.*, 22(5–6): 675–688.

- Mengod, G., Nguyen, H., Le, H., Waeber, C., Lübbert, H. and Palacios, J.M. (1990a) The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. *Neuroscience*, 35: 577–591.
- Mengod, G., Pompeiano, M., Martínez-Mir, M.I. and Palacios, J.M. (1990b) Localization of the mRNA for the 5-HT₂ receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res.*, 524: 139–143.
- Millan, M.J., Girardon, S. and Dekeyne, A. (1999) 5-HT_{2C} receptors are involved in the discriminative stimulus effects of citalopram in rats. *Psychopharmacology*, 142(4): 432–434.
- Miner, L.A., Backstrom, J.R., Sanders-Bush, E. and Sesack, S.R. (2003) Ultrastructural localization of serotonin 2A receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience*, 116(1): 107–117.
- Mnie-Filali, O., Mansari, M.E., Espana, A., Sanchez, C. and Haddjeri, N. (2006) Allosteric modulation of the effects of the 5-HT reuptake inhibitor escitalopram on the rat hippocampal synaptic plasticity. *Neurosci. Lett.*, 395(1): 23–27.
- Moeller, F.G., Schmitz, J.M., Steinberg, J.L., Green, C.M., Reist, C., Lai, L.Y., Swann, A.C. and Grabowski, J. (2007) Citalopram combined with behavioral therapy reduces cocaine use: a double-blind, placebo-controlled trial. *Am. J. Drug Alcohol Abuse*, 33(3): 367–378.
- Moser, P.C., Moran, P.M., Frank, R.A. and Kehne, J.H. (1996) Reversal of amphetamine-induced behaviours by MDL 100,907, a selective 5-HT_{2A} antagonist. *Behav. Brain Res.*, 73(1–2): 163–167.
- Muller, C.P. and Carey, R.J. (2006) Intracellular 5-HT 2C-receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol. Sci.*, 27(9): 455–458.
- Munzar, P., Baumann, M.H., Shoaib, M. and Goldberg, S.R. (1999) Effects of dopamine and serotonin-releasing agents on methamphetamine discrimination and self-administration in rats. *Psychopharmacology*, 141(3): 287–296.
- Munzar, P., Justinova, Z., Kutkat, S.W. and Goldberg, S.R. (2002) Differential involvement of 5-HT(2A) receptors in the discriminative-stimulus effects of cocaine and methamphetamine. *Eur. J. Pharmacol.*, 436(1–2): 75–82.
- Naughton, M., Mulrooney, J. and Leonard, B. (2000) A review of the role of serotonin receptors in psychiatric disorders. *Hum. Psychopharmacol.*, 15(6): 397–415.
- Navailles, S., Moison, D., Cunningham, K.A. and Spampinato, U. (2008) Differential regulation of the mesoaccumbens dopamine circuit by serotonin 2C receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. *Neuropsychopharmacology*, 33(2): 237–246.
- Neisewander, J.L. and Acosta, J.I. (2007) Stimulation of 5-HT_{2C} receptors attenuates cue and cocaine-primed reinstatement of cocaine-seeking behavior in rats. *Behav. Pharmacol.*, 18(8): 791–800.
- Nestler, E.J. (2001) Molecular neurobiology of addiction. *Am. J. Addict.*, 10(3): 201–217.
- Nestler, E.J. (2005) Is there a common molecular pathway for addiction? *Nat. Neurosci.*, 8(11): 1445–1449.
- Neumaier, J.F., Vincow, E.S., Arvanitogiannis, A., Wise, R.A. and Carlezon, W.A., Jr. (2002) Elevated expression of 5-HT_{1B} receptors in nucleus accumbens efferents sensitizes animals to cocaine. *J. Neurosci.*, 22(24): 10856–10863.
- Ni, Y.G. and Miledi, R. (1997) Blockage of 5-HT_{2C} serotonin receptors by fluoxetine (Prozac). *Proc. Natl. Acad. Sci. U.S.A.*, 94(5): 2036–2040.
- NIMH. (2006) An Investigation of the Antidepressant Efficacy of the 5-HT_{2A} Antagonist, M100907, in Combination With Citalopram in Treatment Resistant Depression. *Clinicaltrials.gov*. Available at: <http://clinicaltrials.gov/ct2/show/NCT00070694?intr=M100907&rank=3>. Accessed February 4, 2006.
- Niswender, C.M., Copeland, S.C., Herrick-Davis, K., Emeson, R.B. and Sanders-Bush, E. (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.*, 274(14): 9472–9478.
- Nocjar, C., Roth, B.L. and Pehek, E.A. (2002) Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, 111(1): 163–176.
- O'Brien, C.P. (2005) Anticraving medications for relapse prevention: a possible new class of psychoactive medications. *Am. J. Psychiatry*, 162(8): 1423–1431.
- Office of National Drug Control Policy. (2001) The Economic Costs of Drug Abuse in the United States, 1992–1998 (Publication No. NCJ-190636). Executive Office of the President, Washington, DC.
- Owens, M.J., Morgan, W.N., Plott, S.J. and Nemeroff, C.B. (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J. Pharmacol. Exp. Ther.*, 283(3): 1305–1322.
- Palvimäki, E.P., Majasuo, H., Syvälahti, E. and Hietala, J. (2005) Serotonin 5-HT_{2C} receptor-mediated phosphoinositide hydrolysis in rat choroid plexus after fluoxetine and citalopram treatments. *Pharmacol. Research*, 51(5): 419–425.
- Palvimäki, E.-P., Roth, B.L., Majasuo, H., Laakso, A., Kuoppamäki, M., Syvälahti, E. and Hietala, J. (1996) Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT_{2C} receptor. *Psychopharmacology*, 126(3): 234–240.
- Parker, L.L., Backstrom, J.R., Sanders-Bush, E. and Shieh, B.H. (2003) Agonist-induced phosphorylation of the serotonin 5-HT_{2C} receptor regulates its interaction with multiple PDZ protein 1. *J. Biol. Chem.*, 278(24): 21576–21583.
- Parsons, L.H., Koob, G.F. and Weiss, F. (1995) Extracellular serotonin is decreased in the nucleus accumbens during withdrawal from cocaine self-administration. *Behav. Brain Res.*, 73(1–2): 225–228.
- Pasqualetti, M., Ori, M., Castagna, M., Marazziti, D., Cassano, G.B. and Nardi, I. (1999) Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. *Neuroscience*, 92(2): 601–611.
- Pehek, E.A., Nocjar, C., Roth, B.L., Byrd, T.A. and Mabrouk, O.S. (2006) Evidence for the preferential involvement of 5-HT_{2A} serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. *Neuropsychopharmacology*, 31(2): 265–277.

- Phillipson, O.T. (1979) The cytoarchitecture of the interfascicular nucleus and ventral tegmental area of tsai in the rat. *J. Comp. Neurol.*, 187(1): 85–98.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.*, 23: 163–178.
- Porras, G., Di, M.V., Fracasso, C., Lucas, G., De, D.P., Caccia, S., Esposito, E. and Spampinato, U. (2002) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 311–324.
- Pozzi, L., Acconcia, S., Ceglia, I., Invernizzi, R.W. and Samanin, R. (2002) Stimulation of 5-hydroxytryptamine (5-HT(2C)) receptors in the ventro tegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J. Neurochem.*, 82(1): 93–100.
- Price, R.D., Weiner, D.M., Chang, M.S. and Sanders-Bush, E. (2001) RNA editing of the human serotonin 5-HT_{2C} receptor alters receptor-mediated activation of G13 protein. *J. Biol. Chem.*, 276(48): 44663–44668.
- Quarta, D., Naylor, C.G. and Stolerman, I.P. (2007) The serotonin 2C receptor agonist Ro-60-0175 attenuates effects of nicotine in the five-choice serial reaction time task and in drug discrimination. *Psychopharmacology (Berl.)*, 193(3): 391–402.
- Raymond, J.R., Mukhin, Y.V., Gelasco, A., Turner, J., Collinsworth, G., Gettys, T.W., Grewal, J.S. and Garnovskaya, M.N. (2001) Multiplicity of mechanisms of serotonin receptor signal transduction. *Pharmacol. Ther.*, 92(2–3): 179–212.
- Reid, M.S., Casadonte, P., Baker, S., Sanfilippo, M., Braunstein, D., Hitzemann, R., Montgomery, A., Majewska, D., Robinson, J. and Rotrosen, J. (2005) A placebo-controlled screening trial of olanzapine, valproate, and coenzyme Q10/L-carnitine for the treatment of cocaine dependence. *Addiction*, 100(Suppl. 1): 43–57.
- Reynolds, G.P., Templeman, L.A. and Zhang, Z.J. (2005) The role of 5-HT_{2C} receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 29(6): 1021–1028.
- Rinaldi-Carmona, M., Congy, C., Santucci, V., Simiand, J., Gautret, B., Neliat, G., Labeeuw, B., Le Fur, G., Soubrie, P. and Breliere, J.C. (1992) Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine₂ receptor antagonist. *J. Pharmacol. Exp. Ther.*, 262(2): 759–768.
- Robinson, T.E. and Berridge, K.C. (2003) *Addiction*. *Annu. Rev. Psychol.*, 54: 25–53.
- Roth, B.L. (2006) Psychoactive Drug Screening Program. Case Western Reserve University, Cleveland, OH. Available at: <http://pdsp.cwru.edu/>. Accessed March 21, 2006.
- Rothman, R.B., Blough, B.E. and Baumann, M.H. (2006) Dual dopamine-5-HT releasers: potential treatment agents for cocaine addiction. *Trends Pharmacol. Sci.*, 27(12): 612–618.
- Roy, A., Roy, M. and Smelson, D.A. (1998) Risperidone, ERG and cocaine craving. *Am. J. Addict.*, 7(1): p. 90.
- Sabb, A.L., Vogel, R.L., Welmaker, G.S., Sabalski, J.E., Coupet, J., Dunlop, J., Rosenzweig-Lipson, S. and Harrison, B. (2004) Cycloalkyl[b][1,4]benzodiazepinoindoles are agonists at the human 5-HT_{2C} receptor. *Bioorg. Med. Chem. Lett.*, 14(10): 2603–2607.
- Sanchez, C. (2006) The pharmacology of citalopram enantiomers: the antagonism by R-citalopram on the effect of S-citalopram. *Basic Clin. Pharmacol. Toxicol.*, 99(2): 91–95.
- Sanders-Bush, E., Fentress, H. and Hazelwood, L. (2003) Serotonin 5-HT₂ receptors: molecular and genomic diversity. *Mol. Interventions*, 3(6): 319–330.
- Santoni, J.P. (2007) Sanofi-Aventis' Late Stage R&D Pipeline. Sanofi-Aventis. Available at: http://www.sanofi-aventis.com/Images/20070510_exane_en_tcm23-17719.pdf. Accessed February 4, 2008.
- Sattar, S.P. and Bhatia, S.C. (2003) Olanzapine for cocaine cravings and relapse prevention. *J. Clin. Psychiatry*, 64(8): p. 969.
- Sattar, S.P., Grant, K., Bhatia, S. and Petty, F. (2003) Potential use of olanzapine in treatment of substance dependence disorders. *J. Clin. Psychopharmacol.*, 23(4): 413–415.
- Saucier, C., Morris, S.J. and Albert, P.R. (1998) Endogenous serotonin-2A and -2C receptors in Balb/c-3T3 cells revealed in serotonin-free medium-Desensitization and down-regulation by serotonin. *Biochem. Pharmacol.*, 56(10): 1347–1357.
- Schenk, S. (2000) Effects of the serotonin 5-HT(2) antagonist, ritanserin, and the serotonin 5-HT(1A) antagonist, WAY 100635, on cocaine-seeking in rats. *Pharmacol. Biochem. Behav.*, 67(2): 363–369.
- Schlag, B.D., Lou, Z., Fennell, M. and Dunlop, J. (2004) Ligand dependency of 5-HT_{2C} receptor internalization. *J. Pharmacol. Exp. Ther.*, 310(3): 865–870.
- Schmidt, C.J., Black, C.K., Taylor, V.L., Fadaye, G.M., Humphreys, T.M., Nieduzak, T.R. and Sorensen, S.M. (1992) The 5-HT₂ receptor antagonist, MDL 28,133A, disrupts the serotonergic-dopaminergic interaction mediating the neurochemical effects of 3,4-methylenedioxymethamphetamine. *Eur. J. Pharmacol.*, 220: 151–159.
- Schmidt, C.J. and Fadaye, G.M. (1995) The selective 5-HT_{2A} receptor antagonist, MDL 100,907, increases dopamine efflux in the prefrontal cortex of the rat. *Eur. J. Pharmacol.*, 273: 273–279.
- Sekine, Y., Ouchi, Y., Takei, N., Yoshikawa, E., Nakamura, K., Futatsubashi, M., Okada, H., Minabe, Y., Suzuki, K., Iwata, Y., Tsuchiya, K.J., Tsukada, H., Iyo, M. and Mori, N. (2006) Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Arch. Gen. Psychiatry*, 63(1): 90–100.
- Serretti, A., Drago, A. and De, R.D. (2007) HTR2A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. *Curr. Med. Chem.*, 14(19): 2053–2069.
- Setola, V., Dukat, M., Glennon, R.A. and Roth, B.L. (2005) Molecular determinants for the interaction of the valvulopathic anorexigen norfenfluramine with the 5-HT_{2B} receptor. *Mol. Pharmacol.*, 68(1): 20–33.

- Silverstone, P.H. and Cowen, P.J. (1994) The 5-HT₃ antagonist, BRL 46470 does not attenuate *m*-chlorophenylpiperazine (*m*CPP)-induced changes in human volunteers. *Biol. Psychiatry*, 36: 309–316.
- Smelson, D.A., Losonczy, M.F., Davis, C.W., Kaune, M., Williams, J. and Ziedonis, D. (2002) Risperidone decreases craving and relapses in individuals with schizophrenia and cocaine dependence. *Can. J. Psychiatry*, 47(7): 671–675.
- Smelson, D.A., Williams, J., Ziedonis, D., Sussner, B.D., Losonczy, M.F., Engelhart, C. and Kaune, M. (2004) A double-blind placebo-controlled pilot study of risperidone for decreasing cue-elicited craving in recently withdrawn cocaine dependent patients. *J. Subst. Abuse Treat.*, 27(1): 45–49.
- Smelson, D.A., Ziedonis, D., Williams, J., Losonczy, M.F., Williams, J., Steinberg, M.L. and Kaune, M. (2006) The efficacy of olanzapine for decreasing cue-elicited craving in individuals with schizophrenia and cocaine dependence: a preliminary report. *J. Clin. Psychopharmacol.*, 26(1): 9–12.
- Soares, B.G., Lima, M.S., Reisser, A.A. and Farrell, M. (2003) Dopamine agonists for cocaine dependence. *Cochrane Database. Syst. Rev.*, (2): p. CD003352.
- Sora, I., Hall, F.S., Andrews, A.M., Itokawa, M., Li, X.F., Wei, H.B., Wichems, C., Lesch, K.P., Murphy, D.L. and Uhl, G.R. (2001) Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc. Natl. Acad. Sci. USA*, 98(9): 5300–5305.
- Sorensen, S.M., Kehne, J.H., Fadayel, G.M., Humphreys, T.M., Ketteler, H.J., Sullivan, C.K., Taylor, V.L. and Schmidt, C.J. (1993) Characterization of the 5-HT₂ receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. *J. Pharmacol. Exp. Ther.*, 266: 684–691.
- Stein, C., Davidowa, H. and Albrecht, D. (2000) 5-HT(1A) receptor-mediated inhibition and 5-HT(2) as well as 5-HT(3) receptor-mediated excitation in different subdivisions of the rat amygdala. *Synapse*, 38(3): 328–337.
- Stolerman, I.P. (1993) Components of drug dependence: reinforcement, discrimination and adaptation. *Biochem. Soc. Symp.*, 59: 1–12.
- Substance Abuse and Mental Health Services Administration. (2005) Overview of Findings from the 2004 National Survey on Drug Use and Health. NSDUH Series H-27, DHHS Publication No. SMA 05-4061. Office of Applied Studies, Rockville, MD.
- Swanson, L.W. (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res. Bull.*, 9: 321–353.
- Szucs, R.P., Frankel, P.S., McMahon, L.R. and Cunningham, K.A. (2005) Relationship of cocaine-induced c-Fos expression to behaviors and the role of serotonin 5-HT_{2A} receptors in cocaine-induced c-Fos expression. *Behav. Neurosci.*, 119(5): 1173–1183.
- Tambour, S. and Quertemont, E. (2007) Preclinical and clinical pharmacology of alcohol dependence. *Fundam. Clin. Pharmacol.*, 21(1): 9–28.
- Teitler, M., Herrick-Davis, K. and Purohit, A. (2002) Constitutive activity of G-protein coupled receptors: emphasis on serotonin receptors. *Curr. Top. Med. Chem.*, 2(6): 529–538.
- Thomsen, W.J., Grottick, A.J., Menzaghi, F., Reyes-Saldana, H., Espitia, S., Yuskina, D., Whelan, K., Martin, M., Morgan, M., Chen, W., Al-Shama, H., Smith, B., Chalmers, D. and Behan, D. (2008) Lorcaserin, a novel selective human 5-HT_{2C} agonist: in vitro and in vivo pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 325(2): 577–587.
- Tomkins, D.M., Joharchi, N., Tampakeras, M., Martin, J.R., Wichmann, J. and Higgins, G.A. (2002) An investigation of the role of 5-HT(2C) receptors in modifying ethanol self-administration behaviour. *Pharmacol. Biochem. Behav.*, 71(4): 735–744.
- Tsao, P. and von Zastrow, M. (2000) Downregulation of G protein-coupled receptors. *Curr. Opin. Neurobiol.*, 10(3): 365–369.
- Tzschentke, T.M. (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict. Biol.*, 12(3–4): 227–462.
- Van Oekelen, D., Luyten, W.H. and Leysen, J.E. (2003) 5-HT_{2A} and 5-HT_{2C} receptors and their atypical regulation properties. *Life Sci.*, 72(22): 2429–2449.
- Vanover, K.E., Weiner, D.M., Makhay, M., Veinbergs, I., Gardell, L.R., Lameh, J., Del Tredici, A.L., Piu, F., Schiffer, H.H., Ott, T.R., Burstein, E.S., Uldam, A.K., Thygesen, M.B., Schlienger, N., Andersson, C.M., Son, T.Y., Harvey, S.C., Powell, S.B., Geyer, M.A., Tolf, B.R., Brann, M.R. and Davis, R.E. (2006) Pharmacological and behavioral profile of N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N'-(4-(2-methylpropyloxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel 5-hydroxytryptamine(2A) receptor inverse agonist. *J. Pharmacol. Exp. Ther.*, 317(2): 910–918.
- Vocci, F.J., Aciri, J. and Elkashef, A. (2005) Medication development for addictive disorders: the state of the science. *Am. J. Psychiatry*, 162(8): 1432–1440.
- Vocci, F.J. and Appel, N.M. (2007) Approaches to the development of medications for the treatment of methamphetamine dependence. *Addiction*, 102(Suppl. 1): 96–106.
- Walsh, S.L. and Cunningham, K.A. (1997) Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. *Psychopharmacology*, 130: 41–58.
- Weiner, D.M., Burstein, E.S., Nash, N., Croston, G.E., Currier, E.A., Vanover, K.E., Harvey, S.C., Donohue, E., Hansen, H.C., Andersson, C.M., Spalding, T.A., Gibson, D.F., Krebs-Thomson, K., Powell, S.B., Geyer, M.A., Hacksell, U. and Brann, M.R. (2001) 5-Hydroxytryptamine_{2A} receptor inverse agonists as antipsychotics. *J. Pharmacol. Exp. Ther.*, 299(1): 268–276.
- Weiner, D.M., Vanover, K.E., Brann, M.R., Meltzer, H.Y. and Davis, R.E. (2003) Psychosis of Parkinson's disease: serotonin 2A receptor inverse agonists as potential therapeutics. *Curr. Opin. Investig. Drugs*, 4(7): 815–819.
- Welmaker, G.S., Nelson, J.A., Sabalski, J.E., Sabb, A.L., Potoski, J.R., Graziano, D., Kagan, M., Coupet, J., Dunlop, J., Mazandarani, H., Rosenzweig-Lipson, S., Sukoff, S. and

- Zhang, M. (2000) Synthesis and 5-hydroxytryptamine (5-HT) activity of 2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5-(6H)ones and 2,3,4,4a,5,6-hexahydro-1H-pyrazino[1,2-a]quinoxalines. *Bioorg. Med. Chem. Lett.*, 10(17): 1991–1994.
- Wikstrom, H.V., Mensonides-Harsema, M.M., Cremers, T.I., Moltzen, E.K. and Arnt, J. (2002) Synthesis and pharmacological testing of 1,2,3,4,10,14b-hexahydro-6-methoxy-2-methyldibenzo[c,f]pyrazino[1,2-a]azep in and its enantiomers in comparison with the two antidepressants mianserin and mirtazapine. *J. Med. Chem.*, 45(15): 3280–3285.
- Willins, D.L., Berry, S.A., Alsayegh, L., Backstrom, J.R., Sanders-Bush, E., Friedman, L. and Roth, B.L. (1999) Clozapine and other 5-hydroxytryptamine-2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine-2A receptors in vitro and in vivo. *Neuroscience*, 91(2): 599–606.
- Winhusen, T.M., Somoza, E.C., Harrer, J.M., Mezinskas, J.P., Montgomery, M.A., Goldsmith, R.J., Coleman, F.S., Bloch, D.A., Leiderman, D.B., Singal, B.M., Berger, P. and Elkashef, A. (2005) A placebo-controlled screening trial of tiagabine, sertraline and donepezil as cocaine dependence treatments. *Addiction*, 100(Suppl. 1): 68–77.
- Winter, J.C. and Rabin, R.A. (1993) Antagonism of the stimulus effects of yohimbine and 8-hydroxy-di-propylaminotetralin. *Pharmacol. Biochem. Behav.*, 44: 851–855.
- Wood, M.D. (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.*, 2(6): 383–387.
- Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M. and Jennes, L. (1995) Comparative localization of serotonin 1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J. Comp. Neurol.*, 351: 357–373.
- Yan, Q., Reith, M.E. and Yan, S. (2000) Enhanced accumbal dopamine release following 5-HT_{2A} receptor stimulation in rats pretreated with intermittent cocaine. *Brain Res.*, 863(1–2): 254–258.
- Yoon, S.J., Pae, C.U., Kim, D.J., Namkoong, K., Lee, E., Oh, D.Y., Lee, Y.S., Shin, D.H., Jeong, Y.C., Kim, J.H., Choi, S.B., Hwang, I.B., Shin, Y.C., Cho, S.N., Lee, H.K. and Lee, C.T. (2006) Mirtazapine for patients with alcohol dependence and comorbid depressive disorders: a multicentre, open label study. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 30(7): 1196–1201.
- Zaniewska, M., McCreary, A.C., Przegalinski, E. and Filip, M. (2007) Effects of the serotonin 5-HT_{2A} and 5-HT_{2C} receptor ligands on the discriminative stimulus effects of nicotine in rats. *Eur. J. Pharmacol.*, 571(2–3): 156–165.
- Zueco Perez, P.L. (2002) Mirtazapine in the treatment of cocaine-dependence in patients with methadone. *Actas. Esp. Psiquiatr.*, 30(6): 337–342.

CHAPTER 17

Pharmacological inhibition of dopamine and serotonin activity blocks spontaneous and cocaine-activated behaviour

Robert J. Carey^{1,*}, Joseph P. Huston² and Christian P. Müller³

¹*Research and Development (151), VA Medical Center and SUNY Upstate Medical University, NY 13210, USA*

²*Institute of Physiological Psychology, University of Düsseldorf, 40225 Düsseldorf, Germany*

³*MRC-SGDP Center, Institute of Psychiatry, King's College London, De Crespigny Park, London, UK*

Abstract: The dopaminergic (DA) and serotonergic (5-HT) systems are modulatory transmitter systems that can influence a wide range of behavioural functions. Psychostimulant drugs increase both DA and 5-HT activity by substance-specific mechanisms and, consequently, can broadly influence behavioural and emotional processes in humans and animals. In this chapter, we examine psychostimulant drug effects from the perspective of DA-5-HT and environmental context interactions and anchor this analysis to changes in spontaneous behaviour. In our consideration of the DA and 5-HT transmitter systems, we focus on pharmacological manipulations that target DA and 5-HT autoreceptors. Autoreceptors provide negative feedback inhibitory control of DA and 5-HT neuronal activity so that pharmacological treatments that act on autoreceptors can regulate DA and 5-HT availability. Since psychostimulant drug effects are linked to DA and 5-HT availability, our analysis focuses on investigations that use autoreceptor pharmacology to unravel the complexity of psychostimulant drug action. The overall findings from the experimental manipulations of autoreceptor pharmacology were then used to discuss issues pertinent to drug development for the treatment of psychostimulant drug addiction.

Keywords: serotonin; dopamine; autoreceptors; locomotor activity; cocaine

Introduction

Spontaneous behavioural activity in a familiar and safe environment is widely used as a behavioural assessment to infer an organism's baseline state of response to stimuli. Unlike most behavioural protocols that entail extensive training procedures and, consequently, introduce numerous variables,

the measure of spontaneous activity does not require training, and, thereby, offers minimal complexity to assess an animal's readiness to respond. Dopaminergic (DA) and serotonergic (5-HT) systems in the brain act in concert and exert a pre-eminent control over behavioural activity, so that spontaneous behavioural activity provides a valuable behavioural measurement to quantify effects of DA and 5-HT system manipulations. Not surprisingly then, psychostimulant drugs, like cocaine and d-amphetamine that increase extracellular DA and 5-HT can also

*Corresponding author. Tel.: (315)-425-4866;
Fax: (315)-472-0019; E-mail: careyr@cnyrc.org

augment spontaneous activity, and, thus, enhance the capacity of response-generating systems (for a review, see Müller et al., 2007). Although, the DA and 5-HT systems interact extensively at several levels, the activity within each system can be controlled independently by neurotransmitter-specific autoreceptors. This chapter provides a review of the pharmacological activation or inhibition of DA and/or 5-HT autoreceptors and how such effects impact upon spontaneous behavioural activity. We utilize this information in order to better understand the effects of psychostimulant drugs.

The dopamine–serotonin interaction in the brain

Dopamine and serotonin are neurotransmitters that modulate virtually all behaviours in vertebrates (Hornykiewicz, 1966; Jacobs et al., 1990; Carlsson, 1993; Jacobs and Fornal, 1993). In particular, they play a key role in regulating spontaneous behavioural activity. The DA projections originate from cell bodies located in the ventral tegmental area (VTA) and the substantia nigra (SN) pars compacta of the mesencephalon. Axons from these neurons project to the prefrontal cortex (PFC), structures of the limbic reward system and the caudate putamen (Carlsson, 1993). Also, there is a low-density but functional DA innervation of the neocortex (Berger et al., 1991; Müller and Huston, 2007). The origin of the DA neuron cell bodies that innervate the neocortex outside the PFC is still a matter of debate (Devoto et al., 2001, 2005; Valentini et al., 2004, 2005). The 5-HT system has its cells of origin predominantly in the dorsal (DRN) and median raphe nuclei (MRN) of the mesencephalon and pons. 5-HT axons project virtually to all brain areas (Jacobs and Azmitia, 1992). In both, the DA and 5-HT systems, the innervation are en passage, so that each axon makes synaptic contact with a vast number of other neurons. There are several important projection areas where the DA and 5-HT neurons overlap with a potential for interaction at several levels. The 5-HT projections from the DRN synapse on DA and GABAergic neurons of the VTA, which in turn send projections to the

nucleus accumbens (Herve et al., 1987; Van Bockstaele et al., 1994; Van Bockstaele and Pickel, 1995). This structure has been long known to have a critical role in reward effects of psychostimulant drugs (Koob et al., 1998; McBride et al., 1999). Reciprocally, there is a significant influence of the DA efferents on 5-HT projections (Ferre et al., 1994; Adell et al., 2002). While DA D2-receptors (D2-Rs) in the DRN modulate 5-HT activity at cell body level, they are not involved in the regulation of 5-HT release at terminal level in the striatum. 5-HT projections to the VTA control the firing of DA and non-DA neurons via 5-HT_{1A}-receptors (5-HT_{1A}-R). The local application of 5-HT or the 5-HT_{1A}-R agonist 8-OH-DPAT into the VTA, and the systemic application of the 5-HT_{1A}-R agonist, flesinoxan, was shown to increase the firing rate and burst firing of the VTA DA cells (Arborelius et al., 1993; Pessia et al., 1994; Lejeune and Millan, 1998) and to increase DA release in the Nac (Guan and McBride, 1989). These results implicate post-synaptic 5-HT_{1A}-Rs specifically in DA reward systems. The effect of flesinoxan was abolished by treatment with the selective 5-HT_{1A}-R antagonist, WAY 100635, which by itself, had no effect on the basal firing patterns (Lejeune and Millan, 1998). The release of DA in the striatum, the Nac and the frontal cortex (FC), which are terminal areas of the nigrostriatal, mesolimbic and mesocortical DA projections, respectively, was also increased by the local application in these areas of the 5-HT_{1A}-R agonists, 8-OH-DPAT and ipsapirone (Benloucif and Galloway, 1991; Benloucif et al., 1993; Golembiowska and Wedzony, 1993; Nomikos et al., 1996; Ago et al., 2003). In the VTA, 5-HT_{1A}-Rs are not exclusively localized on DA neurons, as they were also found on non-DA neurons and glia (Doherty and Pickel, 2001). In contrast to DA neurons and GABAergic interneurons that are depolarized by 5-HT, putative GABAergic projection neurons are hyperpolarized by 5-HT. These polarization effects of 5HT can be blocked by the 5-HT_{1A}-R antagonist NAN-190, findings indicative of 5-HT_{1A}-R mediated mechanisms (Cameron and Williams, 1994). On the other hand, an attenuation of the 5-HT innervation of the VTA DA neurons occurs with a local

application of 8-OH-DPAT into the DRN, thereby, activating 5-HT_{1A}-autoreceptors and suppressing 5-HT neurons in the DRN. This suppression of DRN 5-HT activity results in a decrease of extracellular DA levels in the Nac (Yoshimoto and McBride, 1992). Thus, activation of 5-HT_{1A} autoreceptors can lead to a decrease in activity of the reward-related DA neurons in the Nac. In general, 5-HT facilitates DA activity via 5-HT_{1A}-Rs at the somatodendritic level and at the terminal level of the DA projections to basic structures important for behavioural activation and reward mechanisms. In this way, an increase in the 5-HT activity can interact with DA to potentiate a concurrent increase in DA activity. This finding again indicates the influence of 5-HT and, specifically, the 5-HT_{1A} post-synaptic receptor sites in DA areas important for behavioural activation and reward. This finding is also of a critical importance in understanding the addictive potential of psychostimulant drugs that can increase the activity of both DA and 5-HT. In this way, an increase in the 5-HT activity can interact with DA to potentiate the concurrent DA activity.

Blocking DA and 5-HT activity by autoreceptor activation

The activity of the DA and 5-HT neurons in the brain is under the control of inhibitory autoreceptors. Not surprisingly, DA and 5-HT autoreceptors show a high affinity for their respective transmitters. In multi-receptor environments, i.e., when several transmitter receptors are co-localized, these autoreceptors are extremely sensitive to changes in extracellular transmitter concentrations. Their localization at the soma and dendrites of the neuron and at the pre-synaptic terminal sites provides the negative feedback needed to protect a neuron from excessive firing and, in addition, to prevent excessive transmitter release at the post-synaptic receptor sites. In the DA system, somatodendritic as well as pre-synaptic autoreceptors are of the D2-receptor type (Missale et al., 1998). Apomorphine has long been known to be a selective DA autoreceptor agonist (Aghajanian and Bunney, 1973; Di Chiara et al., 1977). In general,

D2 agonists, such as apomorphine, quinpirole or 7-OHDPAT, at low doses can preferentially stimulate D2 autoreceptors and this stimulation is manifested in behaviour by a profound decrease in spontaneous and stimulus-evoked activity (Depoortere et al., 1996). D2 receptors are also present in post-synaptic receptor sites but with a lower level of sensitivity to D2 agonists.

In the 5-HT system, somatodendritic autoreceptors are 5-HT_{1A}-Rs, while pre-synaptic autoreceptors are of the 5-HT_{1B}-R type (Hamon et al., 1990; Barnes and Sharp, 1999; Hoyer et al., 2002). Within the brain, two principal types of 5-HT_{1A}-Rs can be distinguished: the 5-HT_{1A}-autoreceptors and post-synaptic 5-HT_{1A}-Rs. It was shown that the 5-HT_{1A}-R is the inhibitory autoreceptor at the soma and dendrites of the 5-HT neurons in the raphe nuclei (Gozlan et al., 1983; Riad et al., 2000). Post-synaptic 5-HT_{1A}-Rs and 5-HT_{1A}-autoreceptors were shown to possess different properties despite a similar radioligand binding profile (Blier et al., 1993a, b). In the raphe nuclei, 5-HT_{1A}-Rs are localized somatodendritically at 5-HT neurons (Gozlan et al., 1983; Verge et al., 1985; Riad et al., 2000). Their localization is mostly extra-synaptic at the plasma membrane, supporting the idea of a volume transmission activation of these receptors (Agnati et al., 1995; Zoli et al., 1998; Bunin and Wightman, 1999). The source of 5-HT_{1A}-autoreceptor activation is 5-HT released from neurons within one raphe nucleus, or from 5-HT neurons projecting from other raphe nuclei. 5-HT is released by exocytosis. However, approximately 10–30% of the released 5-HT is released from a non-vesicular pool (Adell et al., 2002). Several studies found a high-5-HT_{1A}-R reserve for the inhibition of DRN cell firing and 5-HT synthesis activity (Meller et al., 1990; Cox et al., 1993). This allows a short-term increase in 5-HT_{1A}-R activation without new protein synthesis at the somatodendritic level of 5-HT neurons. A large number of studies consistently found that the pharmacological stimulation of the 5-HT_{1A}-Rs inhibits 5-HT cell firing in the raphe nuclei, and reduces 5-HT synthesis and 5-HT release in the raphe nuclei and in terminal areas of the DRN and MRN projections (Van der Maelen et al., 1986; Sprouse and Aghajanian, 1987, 1988; Hjorth and

Magnusson, 1988; Hutson et al., 1989; Blier et al., 1990; Invernizzi et al., 1991; Bonvento et al., 1992; Yoshimoto and McBride, 1992; Kreiss and Lucki, 1994; Casanovas and Artigas, 1996; Ago et al., 2003). Autoreceptor-mediated inhibitory effects on raphe nuclei can be blocked by 5-HT_{1A}-R antagonists (Martin et al., 1999).

The effect of systemic application of 5-HT_{1A}-R agonists mimics the effects of 5-HT autoreceptor stimulation on extracellular 5-HT levels by decreasing 5-HT in a regionally specific manner (Sharp et al., 1989; Sharp and Hjorth, 1990; Chen and Reith, 1995; Casanovas et al., 1997). Importantly, the ability of 8-OH-DPAT to suppress DRN cell firing, and the ability of the 5-HT_{1A}-R antagonists, spiperone and WAY 100635, to increase DRN cell firing, varies considerably with the behavioural state and the baseline firing activity of the 5-HT cells in a behaving animal (Fornal et al., 1994, 1996). 8-OH-DPAT was most effective in blocking DRN cell firing during low levels of arousal, when spontaneous cell activity was low (drowsiness). In contrast, the 5-HT_{1A}-R antagonist-induced increase in 5-HT activity was most pronounced during the awake, alert state, when basal cell firing was at a high level. Conversely, 5-HT_{1A}-R antagonist effects on cell firing were blunted when animals became drowsy or were asleep (Fornal et al., 1994, 1996). Long-term stimulation (for 14 days) of DRN 5-HT_{1A}-Rs can lead to a desensitization and internalization of the autoreceptors, which results in an attenuated suppression of 5-HT neuron firing frequency (Blier and de Montigny, 1987; Blier et al., 1998; Riad et al., 2001). Such a manipulation in relation to psychostimulant drug use (e.g., cocaine), would serve to enhance the cocaine's indirect potentiation of 5-HT activity, since an increase of extracellular 5-HT would produce less negative feedback inhibition in the 5-HT neurons, thereby, allowing greater 5-HT release. This kind of an effect relates to the importance of understanding the possible contribution of prior drug history to individual differences in response to drugs, such as amphetamine and cocaine. Thus, 5-HT_{1A}-autoreceptors in the raphe nuclei control the firing of 5-HT neurons and, in this way, regulate the 5-HT activity in the terminal regions of the 5-HT projections (Stamford et al., 2000).

Attenuated DA and 5-HT function and spontaneous behaviour

Basal spontaneous behavioural activity in rodent models is usually measured in an enclosed arena in which the animal has been given several exposures, so that it becomes acclimated or habituated to the environment. Behavioural activity includes locomotion as well as rearing and grooming. These are typical and easily quantified spontaneous behaviours for rodents in what is essentially a large enclosed open-field empty arena. Many studies provide evidence for an essential contribution of D2-Rs and 5-HT_{1A}-Rs to basal behavioural activity. The pharmacological stimulation of D2-Rs with autoreceptor preferring doses of apomorphine (0.01–0.05 mg/kg) reduced locomotor activity in rats. This effect was paralleled by a decline in the post-mortem ratio of HVA/DA, but not 5-HIAA/5-HT, indicating a DA-selective effect of the treatment (Carey et al., 2004a).

Low doses of the 5-HT_{1A}-R agonist, 8-OH-DPAT (≤ 0.05 mg/kg, s.c.) in rats, which preferentially activate 5-HT_{1A}-autoreceptors, were found to reduce spontaneous locomotor activity, rearing behaviour, grooming activity and the entries into the central zone of the arena in rats (Dekeyne et al., 2000; Carey et al., 2004a, b, 2005a). Within the autoreceptor preferring dose-range, the observed inhibitory effects of the 5-HT_{1A}-R stimulation were dose-dependent: no effects occurred with a dose of 0.01 mg/kg 8-OH-DPAT, while effects were maximal with 0.05 mg/kg. The inhibitory effects of the 5-HT_{1A}-autoreceptor stimulation could be observed after initial treatment, and for up to nine additional days of treatment. This inhibition could be reversed by pre-treatment with the 5-HT_{1A}-R antagonist, WAY 100635 (Carey et al., 2004a, 2005a). The systemic treatment with the 5-HT_{1A}-R antagonist, WAY 100635, at autoreceptor preferring doses (0.01–0.05 mg/kg), did not affect spontaneous behavioural activity in the open field in rats (Carey et al., 2004a, 2005a). Several intra-cerebral drug-injection studies investigated the contribution of 5-HT_{1A}-autoreceptors to the generation of locomotor activity separately in the DRN and MRN. It was found that pharmacological stimulation of the more sensitive 5-HT_{1A}-autoreceptors in

the DRN induced hypo-activity (Elliott et al., 1990; Higgins and Elliott, 1991), whereas, the stimulation of the MRN 5-HT_{1A}-autoreceptors elicited hyper-activity (Hillegaart and Hjorth, 1989; Elliott et al., 1990; Higgins and Elliott, 1991; Shim et al., 1997). These findings are in line with the view that DRN and MRN 5-HT activity may exert opposite influences on certain behaviours (Lechin et al., 2006). It needs to be kept in mind, however, that these experiments may be confounded by variables, such as: (a) the damage done by the intra-cerebral needle; (b) the spread of the drug away from the injection site; (c) the uncertainty about concentration level of the drug at the receptor sites and (d) the possibility of a depolarization block at some receptor sites due to high-drug concentration. For all of these reasons, it is difficult to extrapolate such findings to systemic injections in which the drug is distributed more or less equally to all receptor sites simultaneously.

Systemic doses of the 5-HT_{1A}-R agonist, 8-OH-DPAT (≥ 0.1 mg/kg), sufficient to stimulate pre- and post-synaptic 5-HT_{1A}-Rs, were shown to predominantly enhance locomotor activity (Dourish et al., 1985; Tricklebank et al., 1986; Lucki et al., 1989; Jackson et al., 1998; Müller et al., 2003b). When a treatment is given, which stimulates the autoreceptors and post-synaptic 5-HT_{1A}-R sites, a situation is created in which release of 5-HT is virtually eliminated, so that the post-synaptic 5-HT stimulation becomes restricted to the 5-HT_{1A}-Rs. This effect is in contrast to a low-dose 5-HT_{1A} treatment, which is selective for autoreceptors and results in decreased 5-HT activity without being accompanied by increased 5-HT_{1A} post-synaptic receptor stimulation. Thus, when high doses of 5HT_{1A}-R agonists are used, post-synaptic 5-HT activity is increased, but it is restricted to 5HT_{1A} post-synaptic receptors only. Although there are reports of behavioural inhibition effects of combined pre- and post-synaptic 5-HT_{1A}-R stimulation (Carli et al., 1989; Hillegaart et al., 1989; Mittman and Geyer, 1989; Dekeyne et al., 2000), such findings relate to behavioural baseline factors (Evenden and Ängeby-Möller, 1990). In attempting to evaluate a behavioural effect of a drug, a basic consideration is often disregarded, namely, the inverted U-function of

the drug effect on behaviour. That is, there is an optimum arousal level for behaviour, and treatments or conditions that shift the level (hyper/hypo arousal), can lead to dysfunctional effects. In the case of drug/behavioural interactions, if an animal is in an optimal arousal state, then, a drug that increases arousal can produce hyper-arousal effect that can be expressed as behavioural inhibition. A behavioural inhibition can occur because so many competing behaviours are being simultaneously activated, that directed behavioural actions are not executed. If, on the other hand, the animal is in a state of low-level arousal, then, the same drug treatment would shift arousal level towards the optimal state and, thereby, lead to behavioural stimulation. In this way, the state of arousal is a critical variable in determining the behavioural outcome of a drug treatment and can lead to confusing and, sometimes, contradictory behavioural observations with the same drug treatment.

Separate pharmacological stimulation of D2- and 5-HT_{1A}-autoreceptors can induce equipotent inhibitory effects on spontaneous behaviour. In fact, an almost complete elimination of locomotor activity, rearing and grooming behaviour was observed in rats when D2- and 5-HT_{1A}-autoreceptor stimulation were combined in a dose-dependent way (Carey et al., 2004b). If the 5-HT_{1A}-R antagonist, WAY 100635, is added to this treatment regimen, then, the suppression induced by 8-OHDPAT is removed, so that the remaining partial suppression becomes restricted to the D2 autoreceptor stimulation. Post-mortem neurochemical measurements confirmed that apomorphine selectively reduced only HVA/DA tissue ratio. 8-OH-DPAT alone reduced only 5-HTAA/5-HT tissue ratio, while apomorphine plus 8-OH-DPAT significantly attenuated the DA as well as the 5-HT turnover (Carey et al., 2004a).

Overall, evidence suggests that stimulation of D2 and 5-HT_{1A} autoreceptors can act independently to reduce spontaneous behaviour by selectively reducing DA or 5-HT activity in the terminal areas of the respective projections. In particular, the 5-HT_{1A}-Rs in the DRN, but not in the MRN, seem to mediate the 5-HT effects. The important finding is that, when the activity of both DA and

5-HT systems is pharmacologically blocked, and DA and 5-HT activity is suppressed in terminal areas, spontaneous behaviour is virtually abolished. Altogether, these findings point up the critical role of combined DA and 5-HT activity to the mediation of adaptive behavioural processes.

Attenuated DA and 5-HT function and cocaine-induced behaviour

Intensive research directed to understand the brain mechanisms by which psychostimulants exert their potent influence on behaviour, have revealed that the mesolimbic DA system plays a crucial role (Koob et al., 1998; Wise, 2002). However, several lines of evidence have clearly demonstrated that DA is not the sole mediator of the behavioural effects of psychostimulant drugs (e.g. Pradhan et al., 1978; Morrow and Roth, 1996; Molina et al., 2001; Hall et al., 2004). It was found that the occupation of the dopamine transporter (DAT) by selective DA re-uptake blockers does not correlate with their locomotor-stimulant effects (Newman et al., 1994; Rothman et al., 1992, 2001). Also, the expression of psychostimulant-induced locomotor sensitization can be dissociated from the expression of the sensitization of the DA response in the Nac (Szumlinski et al., 2000a, b). Furthermore, the effects of psychostimulants on transcription-factor gene expression seem to be mediated by a synergistic action of DA and 5-HT. The cocaine-induced increase in striatal zif268 mRNA expression could be only partially mimicked by the selective DAT blocker, mazindol. This effect could be potentiated with the selective serotonin re-uptake inhibitors (SSRI), fluoxetine or citalopram, but not with the noradrenalin transporter (NAT) blocker, desipramine (Bhat and Baraban, 1993). A lesion of the 5-HT system with *p*-chloroamphetamine significantly attenuated the zif268 response to cocaine in the striatum (Bhat and Baraban, 1993). These findings suggest an essential role for 5-HT, both, alone and in interaction with DA in the psychostimulant effects of cocaine (Tran-Nguyen et al., 1999; Müller and Carey, 2006; Müller and Huston, 2006; Müller et al., 2007a). Furthermore,

recent reports (for a review, see Yano and Steiner, 2007) have shown that 5-HT effects on post-synaptic receptors and on gene expression are an integral component of addictive psychostimulant drugs, such as cocaine.

Psychostimulant drugs, such as cocaine, interfere with the re-uptake function of monoamine transporters for 5-HT, DA and NA (Ross and Renyi, 1967, 1969; Koe, 1976). Since re-uptake is the major mechanism for neurotransmitter inactivation, cocaine, by interfering with re-uptake inactivation, rapidly increases not only the extracellular 5-HT, DA and NA levels in the terminal regions, but also in the regions of the cell bodies (Müller et al., 2007a). In somatodendritic regions of DA and 5-HT neurons, the re-uptake blockade by cocaine leads to an activation of inhibitory D2- and 5-HT_{1A}-autoreceptors and a decrease in DA and 5-HT neuronal activity (Pitts and Marwah, 1986, 1987; Lakoski and Cunningham, 1988; Rutter et al., 1995). While this negative feedback inhibition of DA and 5-HT neuronal activity attenuates the further release of DA and 5-HT, the continued re-uptake blockade by cocaine maintains the initial surge in DA and 5-HT. In that the inactivation of the DA and 5-HT surge occurs by means of the much slower metabolic degradation of neurotransmitters, cocaine can produce a sustained elevation in extracellular DA and 5-HT. On the other hand, if activation of the D2- and/or 5-HT_{1A}-autoreceptors occurs prior to cocaine, then, there would be a much lower level of neurotransmitter being subjected to the re-uptake blockade by cocaine. Thus, the increase in the terminal DA and 5-HT increase by cocaine would be diminished. Such an attenuation of the cocaine-induced 5-HT increase was reported after pre-treatment with the 5-HT_{1A}-R agonist 8-OH-DPAT (Müller et al., 2003b). Consistent with these neurochemical findings, behavioural studies showed that pre-treatment with an autoreceptor dose of 8-OHDPAT (0.01–0.05 mg/kg) decreased the behavioural effect of cocaine (Carey et al., 2004a, b). Similarly, the pharmacological stimulation of D2-autoreceptors with a low dose of apomorphine given prior to cocaine administration was shown to attenuate the cocaine-induced increase in locomotor activity in rats (Carey et al., 2004a). Other studies using local

application of 5-HT_{1A}-R ligands into the region of the raphe nuclei also support an inhibitory role of 5-HT_{1A}-autoreceptors in cocaine-induced hyper-locomotion, but, also showed that there are differences in the contribution of several 5-HT_{1A}-autoreceptor populations (Herges and Taylor, 1999a, b; Szumlinski et al., 2004).

Concerning the behavioural stimulant effects of cocaine, it should be noted that higher doses of 5-HT_{1A}-R agonists and antagonists, which target autoreceptors and post-synaptic receptors, were predominantly found to modulate these cocaine effects in other directions. De La Garza and Cunningham (2000) found that stimulation of the 5-HT_{1A}-R with 8-OH-DPAT (0.1–0.2 mg/kg) potentiated cocaine-induced hyper-locomotion. A behavioural fine analysis revealed that this effect was only observed in the periphery of the open-field arena. The authors noted that the effect is on locomotion at the periphery and may be masked when the whole size of the arena was considered, as was the case, e.g., in a study by Przegalinski and Filip (1997) and their report of an inhibitory effect. Subsequent studies also found potentiating effects of 0.2–0.4 mg/kg 8-OH-DPAT on hyper-locomotion induced by cocaine in large and small test arenas in well-habituated animals (Carey et al., 2001, 2002a, b, 2004a; Müller et al., 2003b). An attenuation of the cocaine-induced increase in rearing behaviour was also evident in these studies. Since the drug effects on locomotor activity interact with contextual variables (e.g., size of the arena, pre-test handling and habituation procedures), then non-drug variables need to be considered in assessing behavioural effects of a drug treatment. The drug effects in non-habituated animals may interact with aversive components of the test procedure, such as a large open area of a testing arena (Thiel et al., 1998, 2000). A similar interaction was also reported for cocaine, in which the degree of locomotor activation, but not the degree of suppression of grooming, depends on pre-test experience of the arena (Carey et al., 2005c, d). Differences in the test procedure may, therefore, be a crucial factor in accounting for the different findings of the effects of high doses

of 5-HT_{1A}-R agonists on the acute hyper-locomotor effects of cocaine.

Interestingly, pre-treatment with 5-HT_{1A}-R antagonists at higher doses had, in the majority of studies, the same effects on cocaine-induced behavioural activation as the autoreceptor selective doses. The 5-HT_{1A}-R antagonists, NAN-190 (0.5–2.0 mg/kg) and WAY 100635 (0.4–0.8 mg/kg), attenuated the acute hyper-locomotor and rearing effects of cocaine in rats (King et al., 1993a; Carey et al., 2000, 2001, 2002a; Müller et al., 2002a, b). Herges and Taylor (1998) and Przegalinski and Filip (1997), however, failed to find an influence of WAY 100135 or WAY 100635 on cocaine-induced behavioural activation. In mice, pre-treatment with WAY100635 (0.1 and 1 mg/kg) potentiated cocaine (15 mg/kg)-induced hyper-locomotion (Nakamura et al., 2006). These seemingly complex findings appear explicable by the unusual types of receptor stimulation that occurs when 5HT_{1A}-R agonist/antagonist doses are used, which, both, activate/inhibit 5HT_{1A}-autoreceptors and post-synaptic receptors simultaneously. In the case of a high-dose of a 5-HT_{1A}-R antagonist, such as WAY-1006365, the autoreceptor would be blocked and, thereby, diminish the negative feedback effects of a high-extracellular level of 5-HT. In terminal areas, however, the high dose of 5-HT would permit stimulation of all post-synaptic 5-HT-Rs, except 5-HT_{1A} post-synaptic receptors. Such an imbalance in post-synaptic 5-HT activation would be expected to have complex and unusual behavioural effects. These observations stress the importance of using low-dose levels in which there is a preferential agonism/antagonism of the 5-HT_{1A}-autoreceptors.

While inhibition of DA or 5-HT separately prior to cocaine attenuates the acute behavioural effects of cocaine down to the level of saline-treated animals, a combined DA and 5-HT blockade had even more profound inhibitory effects. The combined D2- and 5-HT_{1A}-R activation with autoreceptor-preferring doses of apomorphine and 8-OH-DPAT, given prior to cocaine, reduced behavioural activity induced by the cocaine treatment to below saline levels, suggesting an additive interaction contribution of both systems to this acute cocaine effects (Carey et al., 2004a, 2005b).

With low-doses of cocaine (≤ 5 mg/kg), the inhibitory effect was persistent over five treatment days. However, when a medium dose of cocaine was tested (10 mg/kg), the inhibitory effect of the combined apomorphine/8-OH-DPAT pre-treatment gradually diminished in the course of five treatment days (Carey et al., 2005b). A detailed behavioural and neurochemical analysis revealed that this loss of effect was not due to cocaine-sensitization or due to tolerance to the 8-OHDPAT plus apomorphine pre-treatment. Instead, it was due to concomitantly induced Pavlovian drug conditioning effects. Indeed, whenever one drug treatment is followed by another more potent drug treatment in a protocol this type of drug pairing is the same as a Pavlovian conditioning paradigm. This important but generally unrecognized dimension of drug-treatment protocol is embedded in studies using drug manipulations to modify psychostimulant drug effects. Consistent with this Pavlovian conditioning consideration, it has been shown that drug cues generated by apomorphine/8-OH-DPAT can be transformed into cocaine conditioned drug stimuli, which then can come to elicit cocaine hyperlocomotor activity after repeated pairings (Carey et al., 2005b, c). Pavlovian conditioning, however, does not affect the acute locomotor effects of cocaine or other psychostimulant drugs, in that conditioning requires repeated pairings to develop. Thus, for acute cocaine behavioural stimulant effects to occur, increases in both the DA and 5-HT transmitter levels are necessary.

In the study of pharmacological manipulations of the 5-HT_{1A} autoreceptors, it is important to differentiate behavioural and neurotransmitter effects. It has been repeatedly demonstrated (e.g., Müller et al., 2002a) that 5-HT autoreceptor antagonists, such as WAY-100635, alter the neurotransmitter profile of cocaine, such that increases in DA are unaffected, but 5-HT increases are enhanced. Thus, the cocaine effect becomes relatively more 5-HT. Furthermore, if the dosage of WAY-100635 is sufficiently high, it also blocks post-synaptic 5-HT_{1A}-Rs. In this circumstance, cocaine increases 5-HT much more than DA, and, in addition, the 5-HT postsynaptic activation increases at all 5-HT receptor sites but

is blocked at the 5-HT_{1A} postsynaptic receptor sites. On the other hand, a 5-HT_{1A} agonist, such as 8-OH-DPAT, would be expected to blunt a cocaine effect on 5-HT activity without altering cocaine-induced DA increase. Thus, pharmacological agonist/antagonist manipulations of autoreceptors can shift cocaine neurotransmitter balance to become either more 5-HT and less DA or more DA and less 5-HT. Given this complex constellation of effects, it is not surprising that diverse behavioural findings have been obtained with pharmacological manipulations of the 5-HT autoreceptors.

Conclusion

It has long been known that the DA as well as the 5-HT systems are crucial for spontaneous and for cocaine-induced behavioural activity. Since the activity of both systems is under tight control of inhibitory autoreceptors, these receptors appear to be interesting pharmacological targets to manipulate spontaneous or pharmacologically induced behavioural activity. The high affinity and sensitivity of these autoreceptors to the endogenous ligands, as well as to D2- or 5-HT_{1A}-R ligands, indicates that ligands can be used to preferentially stimulate these receptors in a systemic approach with very low-autoreceptor preferring doses. Recent studies have demonstrated that D2- as well as 5-HT_{1A}-autoreceptor stimulation inhibits spontaneous behavioural activity to an equal extent. When DA and 5-HT activity are reduced together, spontaneous activity is virtually eliminated. A similar inhibitory effect was found for cocaine-induced increases in behavioural activity. Stimulating DA and 5-HT autoreceptors prior to cocaine, blocked the cocaine-induced increase in activity to an equal extent, and when administered together, the combined DA and 5-HT autoreceptor stimulation further suppressed cocaine stimulant effects. Importantly, using a systemic approach, the effects of the receptor ligands can be very different at higher doses, when, in addition to the autoreceptors, postsynaptic receptors also get involved. The use of these effects as potential treatment for cocaine abuse, however, is

compromised by the observation, that the 5-HT_{1A}-autoreceptor agonism alone, or in combination with D2-autoreceptor agonism, acquires the properties of a conditioned stimulus as a consequence of repeated pairing with cocaine. This effect can counteract the initial inhibitory effect. As such, the involvement of both DA and 5-HT systems in learning processes (Jacobs and Azmitia, 1992; Schultz, 1998; Meneses, 1999), and partially opposite effects at higher doses ranges, may compromise the use of the present findings for pharmacotherapy of locomotor disorders or psychostimulant abuse.

Altogether, the analysis of agonist/antagonist pre-treatment effects on the DA and 5-HT systems and their interactions, as shown by the strategic use of low-dose autoreceptor-preferring levels has important implications for drug development targeting psychostimulant drug addiction. One key consideration lies in understanding and establishing the necessity of involving both the DA and 5-HT systems for any drug development directed at psychostimulant drug addiction. Moreover, this chapter has provided extensive details, showing that pharmacological manipulation of either DA or 5-HT systems, or both, can have complex and, sometimes, unintended consequences, in that both systems contribute critically to vital behavioural processes. Indeed, pharmacological pre-treatments targeting these systems designed to mitigate psychostimulant drug abuse problems may, instead, create additional problems. That is, any time a drug treatment is used in conjunction with a psychostimulant drug, the possibility is created that the drug treatment, itself, may acquire conditioned stimulus properties as a result of Pavlovian-conditioning processes and eventually result in an opposite effect contrary to the intended mitigative effect; i.e., the drug treatment may eventually come to exacerbate the addictive need for a psychostimulant. This issue of Pavlovian conditioning is relevant to drug development in another important way. That is, with long-term use of psychostimulant drugs, Pavlovian conditioning mechanisms can confer conditioned stimulus properties onto a variety of idiosyncratic stimuli. These conditioned stimulus properties are

consigned to memory and can subsequently come to unexpectedly activate a craving for the psychostimulant drug and, thus, induce a relapse. This consideration argues against a strictly pharmacological approach to the treatment of psychostimulant drug addiction and points to the need to formulate treatments in both, a behavioural and neurochemical context.

Acknowledgements

This work was supported by a VA merit review grant (to Robert J. Carey) and by the Deutsche Forschungsgemeinschaft grant HU 306/23-5 (to Joseph P. Huston and Christian P. Müller).

References

- Adell, A., Celada, P., Abellan, M.T. and Artigas, F. (2002) Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res. Rev.*, 39: 154–180.
- Aghajanian, G.K. and Bunney, B.S. (1973) Central dopaminergic neurons: neurophysiological identification and responses to drugs. In: Snyder S. and Usdin E. (Eds.), *Frontiers in Catecholamine Research*. Pergamon, New York, pp. 643–648.
- Agnati, L.F., Zoli, M., Stromberg, I. and Fuxe, K. (1995) Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience*, 69: 711–726.
- Ago, Y., Koyama, Y., Baba, A. and Matsuda, T. (2003) Regulation by 5-HT_{1A} receptors of the in vivo release of 5-HT and DA in mouse frontal cortex. *Neuropharmacology*, 45: 1050–1056.
- Arborelius, L., Chergui, K., Murase, S., Nomikos, G.G., Hook, B.B., Chouvet, G., Hacksell, U. and Svensson, T.H. (1993) The 5-HT_{1A} receptor selective ligands, (R)-8-OH-DPAT and (S)-UH-301, differentially affect the activity of midbrain dopamine neurons. *Naunyn Schmiedeberg Arch. Pharmacol.*, 347: 353–362.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38: 1083–1152.
- Benloucif, S. and Galloway, M.P. (1991) Facilitation of dopamine release in vivo by serotonin agonists — studies with microdialysis. *Eur. J. Pharmacol.*, 200: 1–8.
- Benloucif, S., Keegan, M.J. and Galloway, M.P. (1993) Serotonin-facilitated dopamine release in-vivo — pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 265: 373–377.
- Berger, B., Gaspar, P. and Verney, C. (1991) Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. *Trends Neurosci.*, 14: 21–27.
- Bhat, R.V. and Baraban, J.M. (1993) Activation of transcription factor genes in striatum by cocaine — role of both

- serotonin and dopamine systems. *J. Pharmacol. Exp. Ther.*, 267: 496–505.
- Blier, P. and de Montigny, C. (1987) Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiological studies in the rat brain. *Synapse*, 1: 470–480.
- Blier, P., Lista, A. and de Montigny, C. (1993a) Differential properties of pre- and postsynaptic 5-hydroxytryptamine_{1A} receptors in the dorsal raphe and hippocampus: I. Effect of spiperone. *J. Pharmacol. Exp. Ther.*, 265: 7–15.
- Blier, P., Lista, A. and de Montigny, C. (1993b) Differential properties of pre- and postsynaptic 5-hydroxytryptamine_{1A} receptors in the dorsal raphe and hippocampus: II. Effect of pertussis and cholera toxins. *J. Pharmacol. Exp. Ther.*, 265: 16–23.
- Blier, P., Pineyro, G., el Mansari, M., Bergeron, R. and de Montigny, C. (1998) Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Ann. NY Acad. Sci.*, 861: 204–216.
- Blier, P., Serrano, A. and Scatton, B. (1990) Differential responsiveness of the rat dorsal and median raphe 5-HT systems to 5-HT₁ receptor agonists and *p*-chloroamphetamine. *Synapse*, 5: 120–133.
- Bonvento, G., Scatton, B., Claustre, Y. and Rouquier, L. (1992) Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. *Neurosci. Lett.*, 137: 101–104.
- Bunin, M.A. and Wightman, R.M. (1999) Paracrine neurotransmission in the CNS: involvement of 5-HT. *Trends Neurosci.*, 22: 377–382.
- Cameron, D.L. and Williams, J.T. (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. *J. Neurosci.*, 14: 6763–6767.
- Carey, R., Damianopoulos, E. and DePalma, G. (2000) The 5-HT_{1A} antagonist WAY 100635 can block the low-dose locomotor stimulant effects of cocaine. *Brain Res.*, 862: 242–246.
- Carey, R.J., DePalma, G. and Damianopoulos, E. (2001) Cocaine and serotonin: a role for the 5-HT_{1A} receptor site in the mediation of cocaine stimulant effects. *Behav. Brain Res.*, 126: 127–133.
- Carey, R.J., DePalma, G. and Damianopoulos, E. (2002a) 5-HT_{1A} agonist/antagonist modification of cocaine stimulant effects: implications for cocaine mechanisms. *Behav. Brain Res.*, 132: 37–46.
- Carey, R.J., DePalma, G. and Damianopoulos, E. (2002b) 8-OHDPAT effects upon cocaine unconditioned and conditioned behaviors. A role for drug stimulus effects. *Pharmacol. Biochem. Behav.*, 72: 171–178.
- Carey, R.J., DePalma, G., Damianopoulos, E., Müller, C.P. and Huston, J.P. (2004a) The 5-HT_{1A} receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. *Psychopharmacology*, 177: 46–54.
- Carey, R.J., DePalma, G., Damianopoulos, E., Hopkins, A., Shanahan, A., Müller, C.P. and Huston, J.P. (2004b) Dopaminergic and serotonergic autoreceptor stimulation effects are equivalent and additive in the suppression of spontaneous and cocaine induced locomotor activity. *Brain Res.*, 1019: 134–143.
- Carey, R.J., DePalma, G., Damianopoulos, E., Shanahan, A., Müller, C.P. and Huston, J.P. (2005a) Evidence that the 5-HT_{1A} autoreceptor is an important pharmacological target for the modulation of cocaine behavioral stimulant effects. *Brain Res.*, 1034: 162–171.
- Carey, R.J., DePalma, G., Damianopoulos, E., Shanahan, A., Müller, C.P. and Huston, J.P. (2005b) Pharmacological inhibition of DA- and 5-HT activity blocks spontaneous and cocaine-activated behavior: reversal by chronic cocaine treatment. *Brain Res.*, 1047: 194–204.
- Carey, R.J., DePalma, G., Damianopoulos, E. and Shanahan, A. (2005c) Stimulus gated cocaine sensitization: interoceptive drug cue control of cocaine locomotor sensitization. *Pharmacol. Biochem. Behav.*, 82: 353–360.
- Carey, R.J., DePalma, G. and Damianopoulos, E. (2005d) Acute and chronic cocaine behavioral effects in novel versus familiar environments: open-field familiarity differentiates cocaine locomotor stimulant effects from cocaine emotional behavioral effects. *Behav. Brain Res.*, 158: 321–330.
- Carli, M., Prontera, C. and Samanin, R. (1989) Effect of 5-HT_{1A} agonists on stress-induced deficit in open field locomotor activity of rats: evidence that this model identifies anxiolytic-like activity. *Neuropharmacology*, 28: 471–476.
- Carlsson, A. (1993) Thirty years of dopamine research. *Adv. Neurol.*, 60: 1–10.
- Casanovas, J.M. and Artigas, F. (1996) Differential effects of ipsapirone on 5-hydroxytryptamine release in the dorsal and median raphe neuronal pathways. *J. Neurochem.*, 67: 1945–1952.
- Casanovas, J.M., Lesourd, M. and Artigas, F. (1997) The effect of the selective 5-HT_{1A} agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. *Br. J. Pharmacol.*, 122: 733–741.
- Chen, N.H. and Reith, M.E. (1995) Monoamine interactions measured by microdialysis in the ventral tegmental area of rats treated systemically with (+/–)-8-hydroxy-2-(di-*n*-propylamino) tetralin. *J. Neurochem.*, 64: 1585–1597.
- Cox, R.F., Meller, E. and Waszczak, B.L. (1993) Electrophysiological evidence for a large receptor reserve for inhibition of dorsal raphe neuronal firing by 5-HT_{1A} agonists. *Synapse*, 14: 297–304.
- De La Garza, R.D. and Cunningham, K.A. (2000) The effects of the 5-hydroxytryptamine_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin on spontaneous activity, cocaine-induced hyperactivity and behavioral sensitization: a microanalysis of locomotor activity. *J. Pharmacol. Exp. Ther.*, 292: 610–617.
- Dekeyne, A., Brocco, M., Adhumeau, A., Gobert, A. and Millan, M.J. (2000) The selective serotonin (5-HT)_{1A} receptor ligand, S15535, displays anxiolytic-like effects in the social interaction and Vogel models and suppresses

- dialysate levels of 5-HT in the dorsal hippocampus of freely-moving rats — a comparison with other anxiolytic agents. *Psychopharmacology*, 152: 55–66.
- Depoortere, R., Perrault, G. and Sanger, D.J. (1996) Behavioural effects in the rat of the putative dopamine D3 receptor agonist 7-OH-DPAT: comparison with quinpirole and apomorphine. *Psychopharmacology*, 124: 231–240.
- Devoto, P., Flore, G., Pani, L. and Gessa, G. (2001) Evidence for co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex. *Mol. Psychiatr.*, 6: 657–664.
- Devoto, P., Flore, G., Saba, P., Fa, M. and Gessa, G.L. (2005) Stimulation of the locus coeruleus elicits noradrenaline and dopamine release in the medial prefrontal and parietal cortex. *J. Neurochem.*, 92: 368–374.
- Di Chiara, G., Porceddu, M.L., Fratta, W. and Gessa, G.L. (1977) Postsynaptic receptors are not essential for dopaminergic feedback regulation. *Nature*, 267: 270–272.
- Doherty, M.D. and Pickel, V.M. (2001) Targeting of serotonin1A receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain. *J. Comp. Neurol.*, 433: 390–400.
- Dourish, C.T., Hutson, P.H. and Curzon, G. (1985) Characteristics of feeding induced by the serotonin agonist “8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT). *Brain Res. Bull.*, 15: 377–384.
- Elliott, P.J., Walsh, D.M., Close, S.P., Higgins, G.A. and Hayes, A.G. (1990) Behavioural effects of serotonin agonists and antagonists in the rat and marmoset. *Neuropharmacology*, 29: 949–956.
- Evenden, J.L. and Ångeby-Möller, K. (1990) Effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) on locomotor activity and rearing of mice and rats. *Psychopharmacology*, 102: 485–491.
- Ferre, S., Cortes, R. and Artigas, F. (1994) Dopaminergic regulation of the serotonergic raphe-striatal pathway — microdialysis studies in freely moving rats. *J. Neurosci.*, 14: 4839–4846.
- Fornal, C.A., Marrosu, F., Metzler, C.W., Tada, K. and Jacobs, B.L. (1994) Effects of the putative 5-hydroxytryptamine(1A) antagonists BMY-7378, NAN-190 and (–)-propranolol on serotonergic dorsal raphe unit-activity in behaving cats. *J. Pharmacol. Exp. Ther.*, 270: 1359–1366.
- Fornal, C.A., Metzler, C.W., Gallegos, R.A., Veasey, S.C., McCreary, A.C. and Jacobs, B.L. (1996) WAY-100635, a potent and selective 5-hydroxytryptamine(1A) antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. *J. Pharmacol. Exp. Ther.*, 278: 752–762.
- Golembiowska, K. and Wedzony, K. (1993) Enhancement by ipsapirone of dopamine release in the rat striatum. *Pol. J. Pharmacol.*, 45: 299–308.
- Gozlan, H., El-Mestikawy, S., Pichat, L., Glowinski, J. and Hamon, M. (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: 3H-PAT. *Nature*, 305: 140–142.
- Guan, X.M. and McBride, W.J. (1989) Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. *Brain Res. Bull.*, 23: 541–547.
- Hall, F.S., Sora, I., Drgonova, J., Li, X.F., Goeb, M. and Uhl, G.R. (2004) Molecular mechanisms underlying the rewarding effects of cocaine. *Ann. NY Acad. Sci.*, 1025: 47–56.
- Hamon, M., Gozlan, H., el Mestikawy, S., Emerit, M.B., Bolanos, F. and Schechter, L. (1990) The central 5-HT1A receptors: pharmacological, biochemical, functional, and regulatory properties. *Ann. NY Acad. Sci.*, 600: 114–129.
- Herges, S. and Taylor, D.A. (1998) Involvement of serotonin in the modulation of cocaine-induced locomotor activity in the rat. *Pharmacol. Biochem. Behav.*, 59: 595–611.
- Herges, S. and Taylor, D.A. (1999a) Modulatory effect of *p*-chlorophenylalanine microinjected into the dorsal and median raphe nuclei on cocaine-induced behavior in the rat. *Eur. J. Pharmacol.*, 374: 329–340.
- Herges, S. and Taylor, D.A. (1999b) Modulation of cocaine-induced locomotor activity, rears and head bobs by application of WAY100635 into the dorsal and median raphe nuclei of the rat. *Naunyn Schmiedebergs Arch. Pharmacol.*, 360: 129–134.
- Herve, D., Pickel, V.M., Joh, T.H. and Beaudet, A. (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine-structure and synaptic input to dopaminergic neurons. *Brain Res.*, 435: 71–83.
- Higgins, G.A. and Elliott, P.J. (1991) Differential behavioural activation following intra-raphé infusion of 5-HT1A receptor agonists. *Eur. J. Pharmacol.*, 193: 351–356.
- Hillegaart, V. and Hjorth, S. (1989) Median raphe, but not dorsal raphe, application of the 5-HT1A agonist 8-OH-DPAT stimulates rat motor activity. *Eur. J. Pharmacol.*, 160: 303–307.
- Hillegaart, V., Wadenberg, M.L. and Ahlenius, S. (1989) Effects of 8-OH-DPAT on motor activity in the rat. *Pharmacol. Biochem. Behav.*, 32: 797–800.
- Hjorth, S. and Magnusson, T. (1988) The 5-HT1A receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn Schmiedebergs Arch. Pharmacol.*, 338: 463–471.
- Hornykiewicz, O. (1966) Dopamine and brain functions. *Pharmacol. Rev.*, 18: 925–964.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71: 533–554.
- Hutson, P.H., Sarna, G.S., O’Connell, M.T. and Curzon, G. (1989) Hippocampal 5-HT synthesis and release in vivo is decreased by infusion of 8-OHDPAT into the nucleus raphe dorsalis. *Neurosci. Lett.*, 100: 276–280.
- Invernizzi, R., Carli, M., Di Clemente, A. and Samanin, R. (1991) Administration of 8-hydroxy-2-(di-*n*-propylamino)tetralin in raphe nuclei dorsalis and medianus reduces serotonin synthesis in the rat brain: differences in potency and regional sensitivity. *J. Neurochem.*, 56: 243–247.
- Jackson, D.M., Wallsten, C.E., Jerning, E., Hu, P.S. and Deveney, A.M. (1998) Two selective 5-HT1A receptor

- antagonists, WAY-100 635 and NDL-249, stimulate locomotion in rats acclimated to their environment and alter their behaviour: a behavioural analysis. *Psychopharmacology*, 139: 300–310.
- Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.*, 72: 165–229.
- Jacobs, B.L. and Fornal, C.A. (1993) 5-HT and motor control. *Trends Neurosci.*, 16: 346–352.
- Jacobs, B.L., Wilkinson, L.O. and Fornal, C.A. (1990) The role of brain serotonin. *Neuropsychopharmacology*, 3: 473–479.
- King, G.R., Joyner, C., Lee, T.H. and Ellinwood, E.H. (1993a) Withdrawal from continuous or intermittent cocaine-effects of NAN-190 on cocaine-induced locomotion. *Pharmacol. Biochem. Behav.*, 44: 253–262.
- Koe, B.K. (1976) Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J. Pharmacol. Exp. Ther.*, 199: 649–661.
- Koob, G.F., Sanna, P.P. and Bloom, F.E. (1998) Neuroscience of addiction. *Neuron*, 21: 467–476.
- Kreiss, D.S. and Lucki, I. (1994) Differential regulation of serotonin (5-HT) release in the striatum and hippocampus by 5-HT1A autoreceptors of the dorsal and median raphe nuclei. *J. Pharmacol. Exp. Ther.*, 269: 1268–1279.
- Lakoski, J.M. and Cunningham, K.A. (1988) Cocaine interaction with central monoaminergic systems: electrophysiological approaches. *Trends Pharmacol. Sci.*, 9: 177–180.
- Lechin, F., Van der Dijs, B. and Hernandez-Adrian, G. (2006) Dorsal raphe vs. median raphe serotonergic antagonism. Anatomical, physiological, behavioral, neuroendocrinological, neuropharmacological and clinical evidences: relevance for neuropharmacological therapy. *Prog. Neuropsychopharm. Biol. Psych.*, 30: 565–585.
- Lejeune, F. and Millan, M.J. (1998) Induction of burst firing in ventral tegmental area dopaminergic neurons by activation of serotonin (5-HT)1A receptors: WAY 100,635-reversible actions of the highly selective ligands, flesinoxan and S 15535. *Synapse*, 30: 172–180.
- Lucki, I., Ward, H.R. and Frazer, A. (1989) Effect of 1-(*m*-chlorophenyl)piperazine and 1-(*m*-trifluoromethylphenyl)piperazine on locomotor activity. *J. Pharmacol. Exp. Ther.*, 249: 155–164.
- Martin, L.P., Jackson, D.M., Wallsten, C. and Waszczak, B.L. (1999) Electrophysiological comparison of 5-hydroxytryptamine(1A) receptor antagonists on dorsal raphe cell firing. *J. Pharmacol. Exp. Ther.*, 288: 820–826.
- McBride, W.J., Murphy, J.M. and Ikemoto, S. (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav. Brain Res.*, 101: 129–152.
- Meller, E., Goldstein, M. and Bohmaker, K. (1990) Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. *Mol. Pharmacol.*, 37: 231–237.
- Meneses, A. (1999) 5-HT system and cognition. *Neurosci. Biobehav. Rev.*, 23: 1111–1125.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. and Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, 78: 189–225.
- Mittman, S.M. and Geyer, M.A. (1989) Effects of 5HT-1A agonists on locomotor and investigatory behaviors in rats differ from those of hallucinogens. *Psychopharmacology*, 98: 321–329.
- Molina, P.E., Ahmed, N., Gatley, J., Volkow, N.D. and Abumrad, N.N. (2001) l-tryptophan attenuation of the dopaminergic and behavioral responses to cocaine. *Life Sci.*, 69: 1897–1906.
- Morrow, B.A. and Roth, R.H. (1996) Serotonergic lesions alter cocaine-induced locomotor behavior and stress-activation of the mesocorticolimbic dopamine system. *Synapse*, 23: 174–181.
- Müller, C.P. and Carey, R.J. (2006) Intracellular 5-HT2C-receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol. Sci.*, 27: 455–458.
- Müller, C.P., De Souza-Silva, M.A., DePalma, G., Tomaz, C., Carey, R.J. and Huston, J.P. (2002a) The selective serotonin1A-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. *Behav. Brain Res.*, 134: 337–346.
- Müller, C.P., Carey, R.J., de Souza Silva, M.A., Jocham, G. and Huston, J.P. (2002b) Cocaine increases serotonergic activity in the hippocampus and nucleus accumbens in vivo: 5HT1A-receptor antagonism blocks behavioral but potentiates serotonergic activation. *Synapse*, 45: 67–77.
- Müller, C.P., Carey, R.J., Huston, J.P. and de Souza Silva, M.A. (2007) Serotonin and psychostimulant addiction: focus on 5-HT1A-receptors. *Prog. Neurobiol.*, 81: 133–178.
- Müller, C.P., Carey, R.J., Salloum, J.B. and Huston, J.P. (2003b) Serotonin(1A)-receptor agonism attenuates the cocaine-induced increase in serotonin levels in the hippocampus and nucleus accumbens but potentiates hyperlocomotion: an in vivo microdialysis study. *Neuropharmacology*, 44: 592–603.
- Müller, C.P. and Huston, J.P. (2006) Dissecting region-specific interactions of serotonin receptors with the psychostimulant effects of cocaine. *Trends Pharmacol. Sci.*, 27: 105–112.
- Müller, C.P. and Huston, J.P. (2007) Dopamine activity in the occipital and temporal cortex of rats: dissociating effects of sensory but not pharmacological stimulation. *Synapse*, 61: 254–258.
- Nakamura, S., Ago, Y., Hayashi, A., Itho, S., Kakuda, M., Hashimoto, H., Baba, A. and Matsuda, T. (2006) Modification of cocaine-induced behavioral and neurochemical effects by serotonin1A receptor agonist/antagonist in mice. *Synapse*, 60: 479–484.
- Newman, A.H., Allen, A.C., Izenwasser, S. and Katz, J.L. (1994) Novel 3 alpha-(diphenylmethoxy)tropane analogs: potent dopamine uptake inhibitors without cocaine-like behavioral profiles. *J. Med. Chem.*, 37: 2258–2261.

- Nomikos, G.G., Arborelius, L., Hook, B.B., Hacksell, U. and Svensson, T.H. (1996) The 5-HT_{1A} receptor antagonist (S)-UH-301 decreases dopamine release in the rat nucleus accumbens and striatum. *J. Neural. Transm.*, 103: 541–554.
- Pessia, M., Jiang, Z.G., North, R.A. and Johnson, S.W. (1994) Actions of 5-hydroxytryptamine on ventral tegmental area neurons of the rat in-vitro. *Brain Res.*, 654: 324–330.
- Pitts, D.K. and Marwah, J. (1986) Electrophysiological effects of cocaine on central monoaminergic neurons. *Eur. J. Pharmacol.*, 131: 95–98.
- Pitts, D.K. and Marwah, J. (1987) Cocaine modulation of central monoaminergic neurotransmission. *Pharmacol. Biochem. Behav.*, 26: 453–461.
- Pradhan, S.N., Battacharyya, A.K. and Pradhan, S. (1978) Serotonergic manipulation of the behavioral effects of cocaine in rats. *Commun. Psychopharmacol.*, 2: 481–486.
- Przegalinski, E. and Filip, M. (1997) Stimulation of serotonin (5-HT)_{1A} receptors attenuate the locomotor, but not the discriminative, effects of amphetamine and cocaine in rats. *Behav. Pharmacol.*, 8: 699–706.
- Riad, M., Garcia, S., Watkins, K.C., Jodoin, N., Doucet, E., Langlois, X., El-Mestikawy, S., Hamon, M. and Descarries, L. (2000) Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *J. Comp. Neurol.*, 417: 181–194.
- Riad, M., Watkins, K.C., Doucet, E., Hamon, M. and Descarries, L. (2001) Agonist-induced internalization of serotonin-1A receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). *J. Neurosci.*, 21: 8378–8386.
- Ross, S.B. and Renyi, A.L. (1967) Inhibition of uptake of tritiated catecholamines by antidepressant and related agents. *Eur. J. Pharmacol.*, 2: 181–186.
- Ross, S.B. and Renyi, A.L. (1969) Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. *Eur. J. Pharmacol.*, 7: 270–277.
- Rothman, R.B., Baumann, M.H., Dersch, C.M., Romero, D.V., Rice, K.C., Carroll, F.I. and Partilla, J.S. (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*, 39: 32–41.
- Rothman, R.B., Grieg, N., Kim, A., De Costa, B., Rice, K.C., Carroll, F.I. and Pert, A. (1992) Cocaine and GBR12909 produce equivalent motoric responses at different occupancy of the dopamine transporter. *Pharmacol. Biochem.*, 39: 1135–1142.
- Rutter, J.J., Gundlach, C. and Auerbach, S.B. (1995) Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. *Synapse*, 20: 225–233.
- Schultz, W. (1998) Predictive reward signal of dopamine neurons. *J. Neurophysiol.*, 80: 1–27.
- Sharp, T., Bramwell, S.R. and Grahame-Smith, D.G. (1989) 5-HT₁ agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. *Br. J. Pharmacol.*, 96: 283–290.
- Sharp, T. and Hjorth, S. (1990) Application of brain microdialysis to study the pharmacology of the 5-HT_{1A} autoreceptor. *J. Neurosci. Methods*, 34: 83–90.
- Shim, L., Javadi, J. and Wirtshafter, D. (1997) Dissociation of hippocampal serotonin release and locomotor activity following pharmacological manipulations of the median raphe nucleus. *Behav. Brain Res.*, 89: 191–198.
- Sprouse, J.S. and Aghajanian, G.K. (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse*, 1: 3–9.
- Sprouse, J.S. and Aghajanian, G.K. (1988) Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology*, 27: 707–715.
- Stamford, J.A., Davidson, C., McLaughlin, D.P. and Hopwood, S.E. (2000) Control of dorsal raphe 5-HT function by multiple 5-HT₁ autoreceptors: parallel purposes or pointless plurality? *Trends. Neurosci.*, 23: 459–465.
- Szumliński, K.K., Frys, K.A. and Kalivas, P.W. (2004) Dissociable roles for the dorsal and median raphe in the facilitatory effect of 5-HT_{1A} receptor stimulation upon cocaine-induced locomotion and sensitization. *Neuropsychopharmacology*, 29: 1675–1687.
- Szumliński, K.K., Maisonneuve, I.M. and Glick, S.D. (2000) Differential effects of ibogaine on behavioural and dopamine sensitization to cocaine. *Eur. J. Pharmacol.*, 398: 259–262.
- Szumliński, K.K., McCafferty, C.A., Maisonneuve, I.M. and Glick, S.D. (2000) Interactions between 18-methoxycoronaridine (18-MC) and cocaine: dissociation of behavioural and neurochemical sensitization. *Brain Res.*, 871: 245–258.
- Thiel, C.M., Huston, J.P. and Schwarting, R.K. (1998) Cholinergic activation in frontal cortex and nucleus accumbens related to basic behavioral manipulations: handling, and the role of post-handling experience. *Brain Res.*, 812: 121–132.
- Thiel, C.M., Müller, C.P., Huston, J.P. and Schwarting, R.K. (2000) Auditory noise can prevent increased extracellular acetylcholine levels in the hippocampus in response to aversive stimulation. *Brain Res.*, 882: 112–119.
- Tran-Nguyen, L.T.L., Baker, D.A., Grote, K.A., Solano, J. and Neisewander, J.L. (1999) Serotonin depletion attenuates cocaine-seeking behavior in rats. *Psychopharmacology*, 146: 60–66.
- Tricklebank, M.D., Middlemiss, D.N. and Neill, J. (1986) Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT₁ receptor agonist, RU 24969, in the rat. *Neuropharmacology*, 25: 877–886.
- Valentini, V., Cacciapaglia, F., Frau, R. and Di Chiara, G. (2005) Selective serotonin reuptake blockade increases extracellular dopamine in noradrenalinergic isocortical but not prefrontal areas: dependence on serotonin-1A receptors and independence from noradrenergic innervation. *J. Neurochem.*, 93: 371–382.
- Valentini, V., Frau, R. and Di Chiara, G. (2004) Noradrenaline transporter blockers raise extracellular dopamine in medial prefrontal but not parietal and occipital cortex:

- differences with mianserin and clozapine. *J. Neurochem.*, 88: 917–927.
- Van Bockstaele, E.J., Cestari, D.M. and Pickel, V.M. (1994) Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area — potential sites for modulation of mesolimbic dopamine neurons. *Brain Res.*, 647: 307–322.
- Van Bockstaele, E.J. and Pickel, V.M. (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus-accumbens in rat-brain. *Brain Res.*, 682: 215–221.
- Van der Maelen, C.P., Matheson, G.K., Wilderman, R.C. and Patterson, L.A. (1986) Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a nonbenzodiazepine anxiolytic drug. *Eur. J. Pharmacol.*, 129: 123–130.
- Verge, D., Daval, G., Patey, A., Gozlan, H., Elmestikawy, S. and Hamon, M. (1985) Presynaptic 5-HT autoreceptors on serotonergic cell-bodies and or dendrites but not terminals are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.*, 113: 463–464.
- Wise, R.A. (2002) Brain reward circuitry: insights from unsensed incentives. *Neuron*, 36: 229–240.
- Yano, M. and Steiner, H. (2007) Methylphenidate and cocaine: the same effects on gene regulation? *Trends Pharmacol. Sci.*, 28: 588–596.
- Yoshimoto, K. and McBride, W.J. (1992) Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. *Neurochem. Res.*, 17: 401–407.
- Zoli, M., Torri, C., Ferrari, R., Jansson, A., Zini, I., Fuxe, K. and Agnati, L.F. (1998) The emergence of the volume transmission concept. *Brain Res. Rev.*, 26: 136–147.

CHAPTER 18

Serotonin receptors as potential targets for modulation of nicotine use and dependence

Paul J. Fletcher^{1,2,4,*}, Anh Dzung Lê^{1,3,4} and Guy A. Higgins⁵

¹*Centre for Addiction and Mental Health, Toronto, Ontario, Canada*

²*Department of Psychology, University of Toronto, Toronto, Ontario, Canada*

³*Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada*

⁴*Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada*

⁵*Bioquest Innovations Inc., Toronto, Ontario, Canada*

Abstract: Nicotine use carries considerable health risks and plays a major role in a variety of diseases. Current pharmacological treatments to aid in smoking cessation include nicotine-replacement therapy and non-nicotinic strategies such as bupropion and varenicline. While these treatments benefit some individuals there is still a need for better and more effective treatment strategies. Nicotine is the major psychoactive substance in tobacco. Some behavioural effects of nicotine, including its reinforcing efficacy result in part from activation of mesolimbic dopamine neurons. Modulation of dopamine function is one potential treatment strategy that could treat nicotine dependence. Serotonergic neurons modulate the functioning of dopamine neurons in a complex fashion. Much of this complexity arises from the fact that serotonin (5-HT) exerts its effects through multiple receptor subtypes, some of which even act in apparent functional opposition to each other. This article reviews evidence, primarily from animal experiments, using behavioural procedures relevant to nicotine use on the potential for 5-HT receptors as targets for treating nicotine dependence. The 5-HT_{1A}, 2A, 2C, 3, 4, 6 receptor subtypes have received most experimental attention, with the 5-HT_{1A} and 5-HT_{2C} receptors being the best studied. Several studies have now shown that 5-HT_{1A} receptor antagonists alleviate some of the behavioural signs induced by nicotine withdrawal. Electrophysiological and neurochemical studies show that stimulation of 5-HT_{2C} receptors reduces the function of the mesolimbic dopamine pathway. 5-HT_{2C} receptor agonists block the stimulatory action of nicotine on midbrain dopamine function. They also reduce several behavioural effects of nicotine including its discriminative stimulus properties and reinforcing effects. Although more work remains to be done, 5-HT_{2C} receptor agonists perhaps hold the most promise as potential therapies for smoking cessation.

Keywords: nicotine; serotonin; dopamine; 5-HT_{2C} receptor; 5-HT_{2A} receptor; 5-HT_{1A} receptor; reinforcement and reward; withdrawal

Introduction

Nicotine use and dependence, typically achieved through cigarette smoking, is a major health

problem. Smoking is associated with considerable health risks and plays a major contributory role in a variety of diseases. These include several forms of cancer, cardiovascular disease and respiratory diseases including emphysema and chronic bronchitis (Frishman et al., 2006). Surveys have shown that many current smokers would like to quit this habit; however the success rate at doing so is quite

*Corresponding author. Tel.: + (416)-535-8501, Ext. 4058;
Fax: + (416)-979-6942; E-mail: Paul_Fletcher@camh.net

low (Baillie et al., 1994). Such low success rates are indicative of the insidious nature of nicotine addiction and dependence. Treatment strategies that would help smokers to quit and to remain abstinent once they quit have an important role to play in treating nicotine use and dependence.

Several such strategies have been tested, or are in current use, with at best modest success. One recent estimate indicates that only about 20% of smokers are able to achieve 12 months of abstinence with currently available pharmacotherapies (Schnoll and Lerman, 2006). There is clearly room for development of more efficacious treatments. Pharmaceutical agents for treating smoking can be divided into nicotinic and non-nicotinic strategies (for reviews, see Cryan et al., 2003; George and O'Malley, 2004; Frishman et al., 2006; Siu and Tyndale, 2007). Nicotinic strategies are designed to work by interfering directly with, or substituting for, smoked nicotine. The most widely prescribed nicotinic strategy is nicotine-replacement therapy (NRT), in which nicotine is administered for example orally, via chewing gum or lozenge, transdermally via a patch or intranasally via inhaler (Frishman et al., 2006). A more recent strategy is the recently approved drug varenicline (CHANTIX[®]) which acts as a partial agonist at nicotinic cholinergic receptors (Gonzales et al., 2006; Rollema et al., 2007b).

The second general pharmacological treatment strategy encompasses non-nicotinic agents that do not directly interact with nicotine and nicotinic receptors. Bupropion, initially approved as an antidepressant agent, was approved in 1997 by the FDA for use as a smoking cessation aid under the name ZYBAN[®]. The primary pharmacological action of bupropion is to elevate synaptic levels of the catecholamines noradrenaline and dopamine, as a result of blockade of the transporters for these two neurotransmitters (Ascher et al., 1995). Behaviourally, treatment with bupropion produces statistically significant reductions, compared to placebo control, in nicotine cravings, and withdrawal symptoms during abstinence (Hurt et al., 1997; George and O'Malley, 2004). In two recent head-to-head trials comparing bupropion with varenicline, both treatments were superior to placebo, yet varenicline was superior to

bupropion on the primary measure of continuous smoking abstinence during a 52-week follow-up period (Gonzales et al., 2006; Jorenby et al., 2006). As such, varenicline may represent a significant advance in the treatment of tobacco dependence.

A variety of drugs interacting with other neurotransmitters or neuromodulators have been suggested as potential targets for treating smoking. These include dopamine D3 receptor antagonists, for example SB-277011A (Andreoli et al., 2003; Ross et al., 2007), opioid receptor antagonists (e.g. naltrexone), cannabinoid CB1 receptor antagonists, for example SR141716A (rimonabant; Cohen et al., 2005) and monoamine oxidase type B inhibitors such as selegiline and EVT-302 (Biberman et al., 2003). To a certain degree the rationale for most of this research has been derived from two basic premises. The first is that a principal neurobiological reason for nicotine having such powerful reinforcing effects is that, like many other drugs of abuse, it can indirectly activate the mesolimbic dopamine system (Balfour et al., 2000). The second premise is that these other neuromodulatory systems interact with dopaminergic neurons, and therefore have the capacity to alter the functioning of dopamine pathways, and thus indirectly modulate the effects of nicotine. As shown in this volume, serotonin (5-hydroxytryptamine; 5-HT) exerts a complex modulatory influence over the functioning of dopaminergic systems. As a result, there are good theoretical grounds for considering that manipulating 5-HT function would also affect behavioural responses to nicotine, and that drugs interacting with 5-HT might potentially modify smoking behaviour. The primary objective of this article is to review the evidence for 5-HT receptors as potential targets for modulation of nicotine use and dependence.

Behavioural features of nicotine use and dependence

Tobacco smoking typically begins in adolescence, initially through experimentation, and is determined by multiple factors including the influence of family members, peers, culture and genetics (Mayhew et al., 2000). Nicotine is the primary psychoactive ingredient in tobacco, and studies in

humans and in animals have shown that it is a powerful reinforcer, leading to the dominant view that nicotine is the primary ingredient in tobacco that results in addiction (Stolerman and Jarvis, 1995). While positive reinforcement is a major factor in contributing to nicotine use and dependence, other factors are involved as well.

Cessation of nicotine use can produce a variety of withdrawal signs. In humans, somatic signs associated with nicotine withdrawal include bradycardia, gastrointestinal discomfort and increased appetite. Affective signs of withdrawal include depressed mood, dysphoria, anxiety, irritability, difficulty in concentrating and craving for nicotine (Shiffman and Jarvik, 1976; Hughes et al., 1991). This aversive withdrawal state is a strong motivational factor contributing to the maintenance of smoking with nicotine acting as a negative reinforcer to alleviate or prevent withdrawal signs. The importance of withdrawal to the continuance of nicotine use and dependence is underscored by the observation that the duration and severity of nicotine withdrawal symptoms, including negative affect, may predict relapse to smoking (West et al., 1989; Kenford et al., 1994).

Chronic use of nicotine may produce a number of neuroadaptive changes in aspects of brain function that may impact on dependence and use. For example, changes in nicotinic acetylcholine (ACh) receptor sensitivity have been reported following chronic exposure to nicotine in animals and in human smokers (Breese et al., 1997; Perry et al., 1999; Robinson et al., 2006). These kinds of changes may give rise to tolerance to some of the behavioural effects of nicotine, a phenomenon that in humans may still be apparent for a long period even after smoking cessation (Perkins et al., 2001). In animals, repeated nicotine use can sensitize the functioning of the mesolimbic dopamine system, such that subsequent nicotine exposure produces augmented behavioural and neurochemical responses (Balfour et al., 1998; DiFranza and Wellman, 2007). This type of sensitization has been argued to enhance the attribution of incentive salience to drugs and drug-associated stimuli (Robinson and Berridge, 1993). Environmental stimuli associated with nicotine become powerful conditioned reinforcers and play a major role in

the maintenance of nicotine self-administration and in relapse (Balfour, 2004). Studies in animals have only recently begun to explore the relationship between nicotine and nicotine-associated cues, in the development and maintenance of nicotine-reinforced behaviour, but it is clear that these different classes of stimuli act synergistically to control behaviour (reviewed by Chaudhri et al., 2006).

Aspects of smoking that can be targeted for treatment: role of animal models

Smoking is a multifaceted behaviour involving multiple, different and inter-related processes including positive and negative reinforcement, reward, tolerance, withdrawal and relapse. As such these different behavioural and psychological processes are all potential targets for smoking cessation interventions. Cryan et al. (2003) identified several ways that a treatment could alter aspects of smoking: stopping or reducing smoking intake; reducing the reinforcing value of nicotine; reducing affective and somatic signs of withdrawal; minimizing craving; and reducing the risk of relapse. From the perspective of preclinical studies a number of behavioural tests have been used to measure nicotine use and dependence, and to evaluate the potential for anti-smoking medications in modifying these processes.

Unconditioned effects

Nicotine reliably stimulates locomotor activity in rodents, especially after repeated administration of the drug (Clarke and Kumar, 1983). Therefore measures of locomotor activity can provide a simple test of whether a pharmacological intervention alters the unconditioned effects of nicotine.

Discriminative stimulus properties

Drug discrimination procedures show that nicotine induces a strong interoceptive cue, since rats can be trained to differentially respond based on whether they have been injected with nicotine or

saline (Stolerman et al., 1984). Consequently a drug discrimination assay can be used to determine whether the subjective stimulus properties of nicotine are altered by pharmacological interventions.

Positive reinforcement and reward

Nicotine is reliably self-administered via the intravenous (i.v.) route, and so i.v. self-administration is the primary method for studying the positive reinforcing effects of nicotine (Corrigall and Coen, 1989; Donny et al., 1995). Like other reinforcing agents, nicotine can induce a conditioned place preference (or aversion) to an environment that has been paired with the drug (Fudala et al., 1985; Risinger and Oakes, 1995). Place preference testing can thus be used as a measure of the rewarding, or aversive, properties of non-contingently injected nicotine. Rats will work to stimulate certain parts of the brain via indwelling electrodes; this responding for intracranial self-stimulation (ICSS) provides a good index of activation of reward-related neuronal pathways. ICSS is determined among other factors by the intensity and duration of pulses delivered through the electrode and by current intensity. Manipulation of these parameters can yield psychophysical functions relating the quantity of electrical stimulation to behaviour. Many psychoactive substances shift these psychophysical functions in ways suggestive of altered reward efficacy. In the case of nicotine, it lowers thresholds for ICSS (Huston-Lyons and Kornetsky, 1992; Ivanova and Greenshaw, 1997), suggesting that the rewarding effects of brain stimulation are enhanced by nicotine.

Withdrawal signs

A number of tests for measuring the somatic and affective signs of nicotine withdrawal exist. The simplest way to induce nicotine withdrawal is to administer nicotine chronically, and then either remove the nicotine source or administer nicotinic receptor antagonists. Observational analysis can then be used to record somatic signs of withdrawal. In rodents somatic signs include writhing or abdominal constriction, facial muscle

contractions, eye blinks, ptosis, foot-licking, scratching and escape attempts (Watkins et al., 2000). Behavioural procedures can be used to infer affective motivational states associated with nicotine withdrawal, including exploration on the elevated plus maze, expression of a conditioned place aversion and startle reactivity (Helton et al., 1993; Watkins et al., 2000). Markou and colleagues have described an elegant method for assessing a presumed anhedonic state associated with drug withdrawal, based on the methodology for ICSS. Withdrawal from nicotine leads to an increase in thresholds for ICSS (Epping-Jordan et al., 1998). In other words, during nicotine withdrawal animals respond as though the efficacy of brain stimulation is less rewarding.

Relapse models

In humans the three major factors that can elicit drug craving and trigger relapse to drug seeking are: re-exposure to the drug itself, stress and exposure to cues that have previously been associated with drug use. These stimuli can also trigger reinstatement of drug-seeking behaviour in animals, and this has prompted the development of preclinical models to study factors relating to relapse to drug-seeking behaviour (Shaham et al., 2003; Epstein et al., 2006). The typical experimental design involves a period of drug self-administration followed by a period in which the drug-seeking response is extinguished by ensuring that responses are no longer reinforced by drug delivery. Reinstatement tests can then be conducted using a non-contingent injection of the previously self-administered drug, exposure to a stressor or re-exposure to the drug-associated cues. Reinstatement is then measured as an increased emission of the previously reinforced response relative to a baseline control. The results of experiments using these procedures have uncovered much detail about the mechanisms underlying relapse and reinstatement triggered by different stimuli, yet the validity of these models for testing medications for clinical application is still debated (Epstein et al., 2006; Lerman et al., 2007).

Mechanisms of action of nicotine

The pharmacological effects of nicotine are mediated through nicotinic acetylcholine receptors (nAChR), which are members of the multisubunit, neurotransmitter-gated superfamily of ion channels. Neuronal nicotinic receptors are pentameric structures comprised of distinct protein subunits, of which there are nine α subunits ($\alpha 2$ – $\alpha 10$) and three β subunits ($\beta 2$ – $\beta 4$), each encoded by distinct genes. The five subunits are arranged to form a central pore which is cation permeable. In the rodent central nervous system (CNS), the most extensively characterized and abundant forms are the $\alpha 4\beta 2$ receptor and a homomeric pentamer comprising $\alpha 7$ subunits (Gotti et al., 2007). Other subunit combinations exist in native tissues, including $\alpha 3\beta 4$, $\alpha 3\beta 2$, each having distinct pharmacological and biophysical properties, as well as distinct localization patterns within the CNS (Gotti et al., 2007).

The neuronal nAChR family is complex, and unravelling the role(s) of the distinct subunits in mediating the diverse actions of nicotine has largely depended on two approaches. The first uses gene targeting to generate mice deficient in specific nAChR subunits. The second is a pharmacological approach using drugs selective for specific nAChRs. Evidence derived from both strategies implicate the $\alpha 4\beta 2$ receptor, particularly in the ventral tegmental area (VTA), as playing the critical role in the addictive properties of nicotine, although other subunit combinations contribute (Laviolette and van der Kooy, 2001; Marubio et al., 2003).

The $\alpha 4\beta 2$ subtype is the principal nAChR in CNS subregions such as cortex, striatum and regions of the mesolimbic pathway. The clinical efficacy of varenicline in the treatment of smoking cessation (Gonzales et al., 2006; Jorenby et al., 2006), a drug with a well-defined functional selectivity for the $\alpha 4\beta 2$, at least compared to $\alpha 3\beta 4$, $\alpha 7$ and the $\alpha 1\beta\gamma\delta$ (skeletal muscle) subunits (Rollema et al., 2007a), provides the strongest empirical evidence for the critical role of the $\alpha 4\beta 2$ subunit in nicotine/tobacco use and dependence. As a partial agonist at the $\alpha 4\beta 2$ receptor, its efficacy is hypothesized to be due to limiting

nicotine from activating circuits to an extent responsible for its reinforcing effects, while activating the same circuits to a level sufficient to blunt craving (Rollema et al., 2007b). Pharmacological studies in animals are also consistent with these data. DH β E is selective for the $\beta 2$ subunit and a potent nicotinic antagonist at most heteromeric nAChR subtypes, including the $\alpha 4\beta 2$ subtype, but with low affinity for the $\alpha 7$ receptor. Conversely methyllycaconitine is considered a potent and selective antagonist for the $\alpha 7$ receptor (Grottick et al., 2000b). DH β E reliably decreases nicotine self-administration and blocks the discriminative stimulus properties of nicotine (Watkins et al., 1999, 2000; Shoaib et al., 2000; Grottick et al., 2000b), while the data for methyllycaconitine are less consistent and typically negative (Brioni et al., 1996; Grottick et al., 2000b; Markou and Paterson, 2001). Furthermore, agonists having functional preference for the $\alpha 4\beta 2$ receptor are more likely to have motor stimulant properties, elicit motor sensitization and generalize to nicotine in a discrimination assay compared to $\alpha 7$ selective agonists (Grottick et al., 2000b, c; Cohen et al., 2003; Boess et al., 2007; Rollema et al., 2007a).

Studies in mice deficient in specific nicotine subunits support this pharmacological evidence. For example, $\beta 2$ subunit knockout mice show attenuated nicotine self-administration, and nicotine-induced mesolimbic dopamine release, compared to wild-type controls (Picciotto et al., 1999). Both deficits are restored by targeted re-expression of the $\beta 2$ subunit in the VTA (Maskos et al., 2005). $\beta 2$ knockout mice also failed to acquire a nicotine discrimination (0.4–0.8 mg/kg) that was readily acquired by wild-type controls (Shoaib et al., 2002). The $\alpha 4$ subunit is implicated in nicotine's effects by two contrasting mutant mouse models. Firstly, an $\alpha 4$ knockout line showed a blunted increase in extracellular striatal dopamine levels in response to nicotine (Marubio et al., 2003). Secondly, a mouse line expressing $\alpha 4$ receptors that are hypersensitive to nicotine (Tapper et al., 2004) showed evidence of accelerated nicotine reward and sensitization compared to wild-type controls. In comparison, no deficits in acquisition or expression of a nicotine discrimination have been reported in mice lacking the $\alpha 7$ subtype

(Stolerman et al., 2004). To the best of our knowledge, the propensity of this knockout line to self-administer nicotine has not been reported.

The VTA is a critical locus for the stimulant and reinforcing effects of nicotine. Reavill and Stolerman (1990) reported that injecting nicotine into the VTA, but not the nucleus accumbens (NAc), dorsal hippocampus or thalamus, stimulated locomotor activity. Similarly, Corrigall et al. (1994) reported that infusing DH β E into the VTA, but not the NAc attenuated nicotine self-administration. The findings of Maskos et al. (2005) demonstrating restoration of nicotine self-administration following a targeted re-expression of the β 2 subunit into the VTA, further support this site as an important locus for the reinforcing and stimulant properties of nicotine. Mechanistically, these effects can be directly attributable to nicotine increasing the firing rates of dopamine cells in the VTA, and consequently increasing dopamine release in the NAc. These effects of nicotine are absent, or at least significantly attenuated, in β 2 and α 4 subunit knockout mice (Picciotto et al., 1998; Marubio et al., 2003), and mimicked to some degree by α 4 β 2 agonists such as varenicline and cytisine (Rollema et al., 2007a). High affinity α 4 β 2 receptors are localized both on interneurons utilizing gamma-aminobutyric acid (GABA) as well as dopamine cell bodies of the VTA. Exogenous nicotine, in concentrations comparable to those experienced by smokers, increases the firing of dopamine cell bodies in the VTA in part via a persistent depression of the inhibitory GABAergic inputs to the VTA via an α 4 β 2 mechanism. Simultaneously nicotine enhances an excitatory glutamatergic input to the dopamine cell body through an α 7 mechanism. An NMDA-dependent long-term potentiation may be established at these synapses. The net effect is a shift towards excitation of the dopamine reward system following nicotine exposure (Mansvelder and McGehee, 2000, 2002).

In common with most drugs of abuse, dependence develops to chronic nicotine use, which in human and animal species includes both somatic and affective signs. In nicotine-dependent rats, elevations in ICSS threshold are precipitated by acute injections of DH β E and mecamylamine,

but not methyllycaconitine (Epping-Jordan et al., 1998; Watkins et al., 2000; Markou and Paterson, 2001), suggesting an α 4 β 2 rather than an α 7 mechanism, and again probably localized to the VTA (Brujinzeel and Markou, 2004). Indeed chronic exposure to nicotine is associated with up-regulation of the α 4 β 2 subtype in multiple regions of the CNS (Flores et al., 1992; Perry et al., 1999). Interestingly, studies with nAChR subunit knockout mice implicate β 2, α 5 and α 7 subunits in mediating distinct withdrawal signs (Jackson et al., 2008).

Brain 5-HT systems

Ascending serotonergic neurons originate mainly in the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN). These two nuclei give rise to a diffuse, separable, but partially overlapping innervation of most parts of the forebrain (Tork, 1990). In the context of this review it is important to note the fact that 5-HT projections innervate all dopamine-rich brain areas including cell body regions (the VTA and substantia nigra) and the terminal regions of the NAc, dorsal striatum and prefrontal cortex. Anatomically then, 5-HT containing neurons are well-situated to influence the activity of dopaminergic cells. Serotonin exerts its influence on cellular activity via binding to specific 5-HT receptors. Multiple 5-HT receptor subtypes exist and are heterogeneously distributed throughout the brain (for reviews, see Barnes and Sharp, 1999; Hoyer et al., 2002). These receptors are divided into seven main subclasses or families, termed 5-HT₁–5-HT₇, based on structural and functional characteristics. Some of these receptor classes can be further subdivided: for example, the 5-HT₁ family contains 5-HT_{1A}, 1B, 1D subtypes, and the 5-HT₂ class contains the 5-HT_{2A}, 2B, 2C subtypes. All of the receptor subtypes, with the exception of the 5-HT₃ receptor, are G-protein coupled receptors; the 5-HT₃ receptor is a ligand-gated ion channel. Depending on their neuronal location, 5-HT receptor subtypes may subserve different functions. For example, the 5-HT_{1A} receptor is located post-synaptically in limbic regions as well as on raphe cell bodies, where it

functions as an autoreceptor controlling impulse flow through 5-HT raphe neurons (Hjorth and Magnusson, 1988). Some 5-HT receptor subtypes may function in an opposing manner. Blockade of 5-HT_{2C} receptors enhances certain behavioural effects of cocaine, whereas blockade of 5-HT_{2A} receptors reduces some of the effects of cocaine (McMahon et al., 2001; Fletcher et al., 2002, 2007). These two receptor subtypes also mediate opposing effects on behavioural measures that are linked to impulsivity (Fletcher et al., 2007).

Given the widespread innervation of the brain by 5-HT neurons, the multiplicity of 5-HT receptors and the diversity of signalling pathways coupled to those receptors it is not surprising that 5-HT has been linked to many different behaviours and psychiatric conditions. In particular disturbances in 5-HT function likely play a role in mood and anxiety disorders (Deakin, 1998). The exact nature of these neurochemical disturbances is not understood, although selective serotonin reuptake inhibitors (SSRIs) are effective in treating these affective states, in at least some individuals (Edwards and Anderson, 1999). To the extent that reduced mood, depression and anxiety are associated with smoking and particularly withdrawal from nicotine this suggests a possible link between 5-HT and nicotine dependence that could be targeted for therapeutic intervention with serotonergic drugs.

Effects of SSRIs on smoking behaviour in humans

Several studies have examined the effects of SSRIs (fluoxetine, sertraline and paroxetine) on smoking cessation. Generally, the results have shown that, in the long-term, SSRIs are not beneficial in enhancing abstinence rates or maintaining cessation (Blondal et al., 1999; Killen et al., 2000; Covey et al., 2002). However, more subtle effects have emerged in some studies. Several reports describe a modest short-term benefit of fluoxetine and sertraline during the first 4 weeks of smoking cessation, though this effect is not sustained at longer treatment intervals (Covey et al., 2002; Niaura et al., 2002). The results of one trial indicated that fluoxetine may be more effective in smokers with

some depressive symptoms at the beginning of the treatment period (Hitsman et al., 1999). This same group also reported that fluoxetine, coupled with cognitive behavioural therapy, increased positive affect while reducing negative affect during a period of smoking cessation (Cook et al., 2004). However, the relationship between these effects and abstinence were not reported. Smoking reduction was observed in depressed alcoholics (Cornelius et al., 1999) treated with fluoxetine and this reduction in smoking was significantly associated with a reduction in alcohol consumption. Finally, following the suggested link between smoking cessation, depression and the antidepressant effects of fluoxetine, Spring et al. (2007) investigated the effects of fluoxetine on smoking cessation in individuals with and without a history of major depression. In agreement with some of their earlier work, fluoxetine coupled with behavioural therapy initially enhanced short-term abstinence rates in those smokers with a history of major depression. However, at 6 months follow-up subjects receiving fluoxetine were significantly more likely to be smoking than subjects receiving placebo; this effect was unrelated to previous depression history.

The effects of SSRI treatment on nicotine dependence appear to parallel those observed for alcohol dependence. In type A alcoholics, who generally have fewer childhood risk factors and develop alcohol dependence later in life, SSRI treatment reduced alcohol consumption and craving but only in the short-term (Balldin et al., 1994; Naranjo et al., 1995). In type B alcoholics, characterized by a greater alcohol dependence, concomitant psychiatric conditions and greater 5-HT deficiency (Virkkunen et al., 1994; Fils-Aime et al., 1996) SSRI treatment produced no improvement or worsening of alcohol dependence (Kranzler et al., 1996; Chick et al., 2004).

Given that SSRIs do not have a long-term beneficial effect on smoking cessation, such treatments are not recommended (Hughes et al., 2007). Despite this conclusion however there is still reason to consider that 5-HT may be a useful target for anti-smoking medication, based in part on the premise that SSRIs may not be the optimal strategy for enhancing 5-HT function. Firstly,

SSRIs in general, and fluoxetine in particular, inhibit the activity of cytochrome P450 enzymes, including some that are involved in the metabolism of nicotine (Hemeryck and Belpaire, 2002; Mandrioli et al., 2006). This action of SSRIs may compromise any putative serotonergic modulation of the effects of nicotine. Secondly, the ability of SSRIs to elevate brain 5-HT function occurs gradually, and likely requires the operation of several neuroadaptive processes including down-regulation of somatodendritic 5-HT_{1A} receptors (Blier et al., 1990; de Montigny et al., 1990). In most trials of SSRIs for smoking cessation it is not clear that such an action has been considered in timing the onset of treatment with the smoking cessation programme. Therefore it is difficult to know how putative elevations in 5-HT function may coincide with behavioural outcome measures in these studies. Finally, while SSRIs enhance brain 5-HT function they do so in an indiscriminate fashion, in that 5-HT neurotransmission likely will be enhanced through all of the various receptor subtypes. As noted above there is strong evidence that different 5-HT receptor subtypes may act in an opposing fashion to modulate neuronal activity and behavioural output. In the case of SSRIs the balance between functionally opposite receptor-mediated events may not be altered much from the basal non-drugged state. In order to shift the balance of neurotransmission between opposing receptor activities it may be necessary to selectively activate individual receptor subtypes. Therefore a potentially more effective treatment strategy may be one which targets individual receptors, rather than one that simply acts to elevate extracellular levels of 5-HT.

Individual receptor subtypes and effects of nicotine

In general very little work has been done to investigate the effects of ligands for the various 5-HT receptor subtypes on the behavioural effects of nicotine that are likely to contribute to nicotine use and dependence. The following sections review the available clinical and preclinical evidence, and identify areas of potential future study. The receptors that have received most attention,

or appear to be the most promising, are the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT₃, 5-HT₄ and 5-HT₆ subtypes.

5-HT_{1A} receptors

High densities of 5-HT_{1A} receptors are found in the DRN and MRN where they function as somatodendritic autoreceptors (Marcinkiewicz et al., 1984; Verge et al., 1986; Pompeiano et al., 1992). Thus, activation of raphe 5-HT_{1A} receptors inhibits the firing activity of serotonergic neurons leading to reduced 5-HT release in terminal areas. The 5-HT_{1A} receptor is also expressed post-synaptically in other brain regions including hippocampus, septum, amygdala and cortical regions. The density of 5-HT_{1A} receptors in NAc and dorsal striatum is very low. Given the differential location of 5-HT_{1A} receptors on raphe cell bodies vs. post-synaptic neurons it is important to note that the functional consequence of selective activation of these different receptor populations are very different.

Several studies have investigated the effects of 5-HT_{1A} receptor ligands on reward or reinforcement-related behaviours. Selectively activating somatodendritic receptors by infusing the 5-HT_{1A} receptor agonist 8-OH-DPAT into the raphe nuclei increases a number of motivated behaviours, increases the rewarding efficacy of ICSS and elicits a conditioned place preference for the environment where the infusions were given (Hutson et al., 1986; Fletcher, 1991; Fletcher et al., 1993; Tomkins et al., 1994). All of these effects presumably are mediated by a reduction in serotonergic neurotransmission. Low doses of systemically administered 8-OH-DPAT are likely to exert a suppressant effect on 5-HT neuronal activity that is not offset by post-synaptic 5-HT_{1A} receptor stimulation. At low doses 8-OH-DPAT (0.03–0.3 mg/kg) did not alter the discriminative stimulus properties of nicotine; a slightly higher dose of 8-OH-DPAT (0.5 mg/kg) failed to affect the acute locomotor activating effect of nicotine (Batman et al., 2005). To the best of our knowledge the effects of 5-HT_{1A} receptor agonists on nicotine self-administration or reinstatement have not been examined.

Relatively high doses of systemically injected 8-OH-DPAT, that likely activate post-synaptic 5-HT_{1A} receptors, reduced responding for cocaine on a fixed ratio schedule (Peltier and Schenk, 1993; Homberg et al., 2004), and enhanced responding on a progressive ratio schedule (Homberg et al., 2004). Since the data reported in that paper are for response rates only it is difficult to know whether these behavioural changes results in altered amounts of cocaine taken. However, inconsistent effects of 5-HT_{1A} receptor agonists including gepirone, ipsapirone and buspirone on cocaine self-administration have been reported (Gold and Balster, 1992; Mosner et al., 1997; Homberg et al., 2004). In the case of ipsapirone no effect of this drug on oral self-administration of nicotine was found (Mosner et al., 1997).

Buspirone (BUSPAR[®]) is a 5-HT_{1A} partial agonist with mixed dopamine D2 receptor agonist/antagonist properties (Fulton and Brogden, 1997). It is one of few 5-HT receptor selective agents that has been tested clinically for smoking cessation. Early open-label studies suggested that buspirone increased quit rates and reduced withdrawal symptoms (Gawin et al., 1989; Robinson et al., 1991), but the results from blinded controlled studies are inconsistent. While two such studies suggested that buspirone increased cessation rates (West et al., 1991; Hilleman et al., 1992), another two larger studies reported that buspirone was ineffective (Robinson et al., 1992; Schneider et al., 1996). One study suggested that anxiety level may be an important determinant of buspirone efficacy, with high-anxiety subjects showing higher quit rates than those with low anxiety scores. This effect was relatively short-lived and disappeared once buspirone was discontinued (Cinciripini et al., 1995). One report suggested that buspirone might alleviate some withdrawal symptoms (Hilleman et al., 1992), but this effect was not borne out in other studies. In fact two studies showed that subjects treated with buspirone reported higher cravings than placebo-treated subjects (West et al., 1991; Schneider et al., 1996). Overall then, the inconsistent effects of buspirone in humans, coupled with the inconsistent effects of several 5-HT_{1A} agonists in models of psychomotor stimulant self-administration in

animals suggest that 5-HT_{1A} receptor agonists are not likely to represent an effective treatment for nicotine use and dependence.

The effects of 5-HT_{1A} receptor antagonists on nicotine self-administration or nicotine-induced reinstatement have not been examined. However, the selective 5-HT_{1A} receptor antagonist WAY100635 did attenuate the ability of cocaine to reinstate responding (Schenk, 2000) suggesting that examination of 5-HT_{1A} receptor antagonists in nicotine reinstatement models may be worth considering.

Behavioural studies of 5-HT_{1A} receptor antagonists indicate some potential for these drugs to reduce aspects of nicotine withdrawal. Withdrawal from chronic nicotine treatment enhanced the magnitude of the acoustic startle reflex (Helton et al., 1993) and this response was reduced by the 5-HT_{1A} receptor antagonists WAY100635 and LY426965 (Rasmussen et al., 1997, 2000). The mechanisms by which these effects occur are not known, although it has been suggested that 5-HT_{1A} receptor-mediated functions are enhanced during nicotine withdrawal. LY426965 slightly increased the firing rate of dorsal raphe 5-HT neurons and augmented the ability of fluoxetine to elevate extracellular levels of 5-HT in the hypothalamus (Rasmussen et al., 2000). It is possible then that 5-HT_{1A} receptor blockade produces its behavioural effect on startle reactivity by reversing a 5-HT deficit induced by nicotine withdrawal.

The results of another experiment also suggest that 5-HT_{1A} receptor blockade might alleviate affective effects of nicotine withdrawal. In this study, withdrawal from nicotine increased the threshold for ICSS, and this effect was reversed by a combined treatment with the 5-HT_{1A} receptor antagonist p-MMPI and the SSRI fluoxetine (Harrison et al., 2001). Interestingly, a similar effect was found in rats undergoing withdrawal from amphetamine treatment. As for the results of experiments involving startle reactivity the mechanism of this effect is not known. However, since the combination of 5-HT_{1A} receptor antagonist and fluoxetine markedly increases 5-HT function a correction of a presumed 5-HT deficit is a possibility.

5-HT_{2A} receptors

The 5-HT_{2A} receptor subtype is especially prominent in cortical areas, and is also found in the dopamine-rich areas of the NAc, striatum, VTA and substantia nigra (Pazos et al., 1985; Pompeiano et al., 1994; Bubser et al., 2001). These 5-HT_{2A} receptors appear to be mainly located post-synaptically in relation to 5-HT neuronal terminals. In the prefrontal cortex 5-HT_{2A} receptors are found on pyramidal neurons as well as GABAergic interneurons (Willins et al., 1997; Cornea-Hebert et al., 1999). Within the cell body regions of the VTA and substantia nigra, 5-HT_{2A} receptors are located on both dopaminergic and non-dopaminergic cells (Cornea-Hebert et al., 1999; Doherty and Pickel, 2000; Nocjar et al., 2002). Thus, 5-HT_{2A} receptors are well-situated to modulate dopamine-mediated functions.

Serotonin acting via the 5-HT_{2A} receptor exerts predominantly an excitatory influence on dopaminergic function. The mixed 5-HT_{2A/2C} receptor agonist DOI elevates extracellular levels of dopamine in NAc and prefrontal cortex (Ichikawa and Meltzer, 1995; Yan et al., 2000; Pehek et al., 2001). Blockade of these effects by preferential 5-HT_{2A} receptor antagonists implicates the 5-HT_{2A} receptor in mediating these neurochemical actions of DOI. Some behavioural effects of nicotine are altered by DOI. In rats, DOI did not alter the acute stimulant action of nicotine, but blocked the development of sensitization to the locomotor stimulant effects of repeated nicotine in rats (Olausson et al., 2001). In mice, DOI prevented the initial suppression of locomotion induced by nicotine (Batman et al., 2005). Two reports show that DOI attenuates the discriminative stimulus properties of nicotine (Batman et al., 2005; Zaniowska et al., 2007); these effects of DOI were blocked by 5-HT_{2A} receptor antagonists but not by 5-HT_{2C} receptor antagonists. While these results suggest that 5-HT_{2A} receptor stimulation opposes some of the behavioural effects of nicotine, other studies show that DOI can be quite disruptive to behaviour even at quite low doses (Fletcher et al., 2007). Drug discrimination studies show generalization between DOI and LSD (Arnt, 1989); and activation of the 5-HT_{2A} receptor has long been

recognized as contributor to the induction of hallucinations by LSD and other drugs (Nichols, 2004). Given these considerations, and despite the ability of DOI to reduce some behavioural effects of nicotine, it is difficult to imagine that 5-HT_{2A} receptor agonists could play any role in treating nicotine dependence in humans.

Blockade of 5-HT_{2A} receptors attenuates the rise in extracellular levels of dopamine induced by amphetamine and cocaine (Porras et al., 2002; Auclair et al., 2004). This blunting effect of 5-HT_{2A} receptor blockade on dopamine function is observed also at the behavioural level, with M100907 attenuating the locomotor stimulant effects of cocaine, amphetamine and other psychomotor stimulants (O'Neill et al., 1999; McMahon and Cunningham, 2001; Fletcher et al., 2002). These effects may be mediated in the VTA since local infusion of M100907 into the VTA blocked amphetamine and cocaine-induced locomotion (McMahon et al., 2001; Auclair et al., 2004). Given these findings, and the importance of the VTA for nicotine-induced reinforcement it might be expected that 5-HT_{2A} receptor antagonists would modify some of the behavioural effects of nicotine. However, surprisingly little work has been done in this area. In rats trained to discriminate nicotine from saline, the discriminative stimulus properties of nicotine were not modified by M100907 (Zaniowska et al., 2007) implying that 5-HT_{2A} receptor blockade does not alter the subjective effects of nicotine. To the best of our knowledge there have been no published studies of the effects of 5-HT_{2A} receptor antagonists on nicotine-induced locomotor activity, nicotine self-administration or nicotine reinstatement. Although 5-HT_{2A} receptor antagonists do not alter self-administration of cocaine, M100907 reduced the effect of cocaine to reinstate drug-seeking behaviour (Fletcher et al., 2002). Thus, examination of 5-HT_{2A} receptor blockade in models of nicotine reinstatement might be promising.

5-HT_{2C} receptors

Initially identified as a 5-HT binding site in choroid plexus (Pazos et al., 1984), a much wider CNS distribution of the 5-HT_{2C} receptor is now

known. Autoradiographic and immunohistochemical techniques are beginning to reveal a comprehensive map of 5-HT_{2C} distribution within regions of cortex (piriform, cingulate, prefrontal), limbic system (NAc, amygdala, hippocampus) and mid-brain (VTA, substantia nigra), and the reasonable overlap between 5-HT_{2C} receptor mRNA and receptor-binding site suggests a predominantly post- rather than pre-synaptic localization (Mengod et al., 1990). A growing body of preclinical evidence from electrophysiological, biochemical and behavioural analyses shows that the 5-HT_{2C} receptor is a key mechanism through which 5-HT can influence dopamine systems. Similar to nicotine, a primary locus for this interaction appears to be at the level of the VTA.

Detailed mapping studies of 5-HT_{2C} receptor distribution in the VTA region of the rat demonstrate 5-HT_{2C} receptors located on dopamine cell bodies and GABAergic interneurons (Bubar et al., 2005; Bubar and Cunningham, 2007). Expression levels appear to vary along the rostrocaudal axis, but seem roughly equivalent across both cell types (Bubar and Cunningham, 2007). Electrophysiological studies indicate a direct excitatory effect of microiontophoretically applied 5-HT in the VTA on inhibitory GABA neurons that serve to decrease the firing rate of dopamine neurons upon which they synapse (Johnson et al., 1992; Prisco et al., 1994). Recent studies using receptor subtype selective agonists identify this effect as being mediated through the 5-HT_{2C} receptor. Thus, the moderately selective 5-HT_{2C} receptor agonist Ro 60-0175 (Martin et al., 1998) reduced basal firing rate of VTA dopamine neurons, an effect which was blocked by the selective 5-HT_{2C} receptor antagonist SB242084 (Di Matteo et al., 1999; Gobert et al., 2000). The direct application of SB242084 alone increased the basal firing rate of dopamine neurons in the VTA, implying a tonic inhibitory drive from serotonergic afferents likely originating in the DRN (Gervais and Rouillard, 2000). As might be expected given these electrophysiological findings, 5-HT_{2C} receptor agonists such as Ro 60-0175 reduced dopamine release in NAc and the frontal cortex, while SB242084 increased DA release (Di Matteo et al., 2002).

Given the critical importance of the VTA as a substrate for the acute reinforcing effect of nicotine (as well as other drugs of abuse), these observations provided us with a rationale to examine the effects of Ro 60-0175 on the motor stimulant and reinforcing effects of nicotine and cocaine (Grottick et al., 2000a, 2001; Higgins and Fletcher, 2003). In rats sensitized to nicotine, Ro 60-0175 blocked the hyperactivity induced by acute injection of nicotine; an effect reversed by SB242084 (Grottick et al., 2001). Further experiments revealed that Ro 60-0175, given concomitantly with nicotine, blocked the development of sensitization to the locomotor stimulant effect of nicotine. Ro 60-0175 also reduced nicotine self-administration (Grottick et al., 2001) (Fig. 1). Thus 5-HT_{2C} receptor activation inhibits both the unconditioned stimulant effects, as well as the positive reinforcing effects, of nicotine. Furthermore, tolerance did not seem to develop to the effect of Ro 60-0175 against nicotine-induced hyperactivity. As yet studies have not investigated the impact of 5-HT_{2C} receptor agonists in a model of nicotine relapse, although our observation that Ro 60-0175 attenuates the reinstatement of cocaine responding elicited either by drug-, cue- or stress-related stimuli (Grottick et al., 2000a; Fletcher et al., 2008) would suggest this investigation is warranted. Recently it has been shown that Ro 60-0175 shifts a nicotine discrimination curve to the right, suggesting that a 5-HT_{2C} agonist may also weaken the discriminative stimulus properties of nicotine (Quarta et al., 2007).

Consistent with these behavioural observations, Ro 60-0175 also attenuated nicotine-induced increases in VTA burst firing and concomitant dopamine release in the NAc (Di Matteo et al., 2004; Pierucci et al., 2004) (Fig. 1). Since 5-HT_{2C} receptor activation on the GABAergic interneurons is excitatory (Di Matteo et al., 2002), this may counter the nicotine-induced desensitization of these inhibitory cells.

A recent article (Ji et al., 2006) has shown that the tumour suppressor phosphate and tensin homologue deleted on chromosome 10 (PTEN) regulates 5-HT_{2C} receptor signalling in the VTA region serving to limit agonist-induced phosphorylation of the 5-HT_{2C} receptor. The peptide

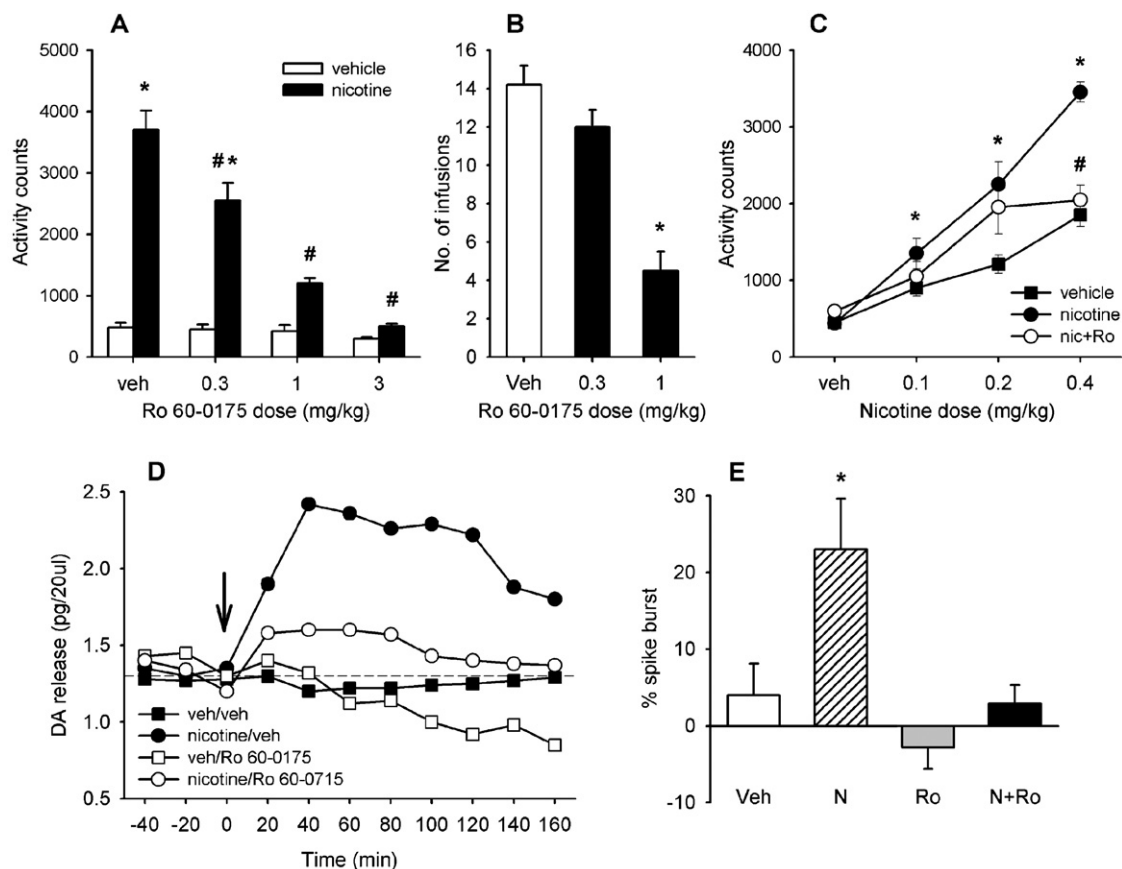


Fig. 1. Summary of evidence supporting a potential role for 5-HT_{2C} receptor agonists as treatments for smoking cessation. (A) Effect of Ro 60-0175 on hyperactivity induced by nicotine (0.4 mg/kg s.c.), in rats previously sensitized to nicotine (10 daily injections of nicotine 0.4 mg/kg s.c.). #*p* < 0.05 vs. vehicle/vehicle pre-treatment, **p* < 0.05 vs. vehicle/nicotine pre-treatment. Redrawn from Fig. 1A and 1B in Grottick et al. (2001). (B) Effect of Ro 60-0175 on nicotine self-administration. Nicotine (0.03 mg/injection) was available for 1 h each day, under an FR5TO 1 min schedule of reinforcement. Redrawn from Fig. 2B in Grottick et al. (2001). (C) Effect of chronic treatment with vehicle, nicotine (0.4 mg/kg s.c.), or nicotine (0.4 mg/kg s.c.) + Ro 60-0175 (1 mg/kg s.c.) on locomotor activity produced by nicotine (0–0.4 mg/kg s.c.). Prior nicotine exposure resulted in an enhanced locomotor response to nicotine (**p* < 0.05), that was blocked by Ro 60-0175 (#*p* < 0.05). Redrawn from Fig. 3 in Grottick et al. (2001). (D) Time course of the effect of acute nicotine (1 mg/kg i.p.) on extracellular dopamine levels in the nucleus accumbens measured by microdialysis. Rats were treated for 10 days with nicotine (1 mg/kg i.p.) prior to the microdialysis experiment. Rats were treated with either vehicle or Ro 60-0175 (1 mg/kg i.p.) 20 min before nicotine (1 mg/kg i.p.) or vehicle (indicated by arrow). Nicotine increased extracellular dopamine release in the nucleus accumbens. This effect was significantly attenuated by Ro 60-0175 pretreatment. Adapted from Fig. 4 in Di Matteo et al. (2004). (E) Effect of nicotine (i.v.) and Ro 60-0175 (0.1 mg/kg i.v.) on the firing pattern of VTA dopamine neurons in rats treated for 10 days with nicotine (1 mg/kg i.p.). The data represent the mean ± SEM difference between the percentage of spikes occurring in bursts during baseline vs. post-drug periods. The data show that nicotine increases burst firing of VTA dopamine neurons, and that this effect is blocked by Ro 60-0175. Data adapted from Table 2 in Pierucci et al. (2004).

Tat-3L4F, which disrupts PTEN coupling with the third intracellular loop of the 5-HT_{2C} receptor, mimicked certain effects of the direct 5-HT_{2C} agonist Ro 60-0175. Both treatments suppressed

the firing rate of dopamine cells, and blocked a nicotine-induced conditioned place preference (Ji et al., 2006). Because other effects of 5-HT_{2C} receptor activation mediated outside the VTA

were not induced by the Tat-3L4F peptide, it has been suggested that inhibitors of PTEN could provide a more selective 5-HT_{2C} receptor-based strategy to treat addiction, including smoking cessation (Ji et al., 2006; Muller and Carey, 2006). Although of significant interest, this research is preliminary and the tractability and feasibility of PTEN inhibition as a target for smoking cessation remains to be established.

As yet it is unknown whether 5-HT_{2C} agonists may blunt the somatic signs associated with nicotine withdrawal, although reports suggesting an antidepressant potential of 5-HT_{2C} agonists (Cryan and Lucki, 2000; Rosenzweig-Lipson et al., 2007), particularly after chronic administration (Moreau et al., 1996) suggests these agents could positively influence the affective state accompanying withdrawal. Again this is worthy of investigation. However, notwithstanding some of these open questions, the ability of a 5-HT_{2C} receptor agonist to block mesolimbic dopamine activation produced by nicotine at the level of the VTA, a property shared with varenicline (Rollema et al., 2007a), suggests that selective 5-HT_{2C} agonists have potential as novel therapies for smoking cessation.

5-HT₃ receptors

Like the nAChR family, the 5-HT₃ receptor is a member of the neurotransmitter-gated superfamily of ion channels, and indeed the two share similarities in terms of primary structure (Barnes and Sharp, 1999). Current evidence seems to suggest that 5-HT₃ receptors comprise a minimum of two subunits, 5-HT_{3A} and 5-HT_{3B} (Davies et al., 1999), although a recent article has proposed the existence of other subunits which if confirmed might account for the functional diversity of 5-HT₃ receptors seen in native tissue preparations and across species (Niesler et al., 2007). Expression studies reveal a fairly broad but low level of 5-HT₃ receptors within the CNS relative to other 5-HT subtypes (Barnes and Sharp, 1999), with the exception of hindbrain nuclei associated with the emetic reflex where expression levels are much higher (Pratt et al., 1990). However, 5-HT₃ receptors are found in regions of the

mesocorticolimbic system, including NAc, cortical subregions and amygdala, although species differences in distribution pattern are evident (Barnes and Sharp, 1999).

Given the structural similarity between 5-HT₃ receptors and nACh receptors, it is perhaps not surprising that nicotine and certain analogues (e.g. epibatidine) interact and have functional (typically inhibitory) effects on 5-HT₃ receptor-mediated currents (Gurley and Lanthorn, 1998; Breiting et al., 2001; Drisdell et al., 2008). However, such interactions occur at high doses of nicotine (e.g. IC₅₀ (–)-nicotine m5-HT₃ = 32 μM; Gurley and Lanthorn, 1998) and at present there is no reliable evidence to suggest that interactions at the 5-HT₃ receptor may account for any pharmacological effects of nicotine.

Highly selective antagonists for the 5-HT₃ receptor emerged in the late 1980s, including MDL72222, ondansetron, tropisetron (ICS205-930) and granisetron. Early observations suggested that these drugs dampened the increased mesolimbic dopamine tone induced by various drugs of abuse (amphetamine, morphine, nicotine), or by direct VTA stimulation such as that produced by local infusions of the neurokinin agonist DiMeC7 (Costall et al., 1987; Carboni et al., 1989; Hagan et al., 1990). Similarly 5-HT₃ receptor antagonists blocked the acquisition of a conditioned place preference induced by nicotine (Carboni et al., 1988). However, in tests of nicotine self-administration, nicotine discrimination, nicotine-induced hyperlocomotion and nicotine-induced reductions of ICSS thresholds, 5-HT₃ receptor antagonists have failed to alter the effects of nicotine (Schechter and Meehan, 1992; Corrigan and Coen, 1994; Arnold et al., 1995; Ivanova and Greenshaw, 1997).

Early data with 5-HT₃ receptor antagonists also suggested that these agents attenuate some affective signs of drug withdrawal following chronic treatment with various abused drugs, including nicotine (Costall et al., 1989, 1990). Suzuki et al. (1997) have also reported that ondansetron blocks the development of a place aversion paired with a nicotine withdrawal state precipitated by mecamylamine treatment, which is consistent with the earlier observations of Costall and coworkers.

However, to the best of our knowledge there are no data to suggest that 5-HT₃ receptor antagonists influence somatic signs of nicotine withdrawal.

Based on the early preclinical results 5-HT₃ receptor antagonists were evaluated clinically for smoking cessation. Two studies found no significant effects of ondansetron treatment on cigarette consumption, abstinence (Zacny et al., 1993; West and Hajek, 1996) or withdrawal severity (West and Hajek, 1996). A further study failed to find evidence for an improvement of withdrawal symptoms in abstinent ex-smokers following granisetron treatment (Hatsukami et al., 2003).

Overall, despite initial promise, there is no reliable preclinical or clinical evidence to suggest that 5-HT₃ receptor antagonists are effective therapies to aid smoking cessation.

5-HT₄ receptors

5-HT₄ receptors are abundant in the dorsal striatum, substantia nigra and NAc; expression of 5-HT₄ receptor in cortical areas is very low. Where 5-HT₄ receptors are found in dopamine-rich regions they appear not to be expressed on dopamine neurons (Patel et al., 1995; Vilario et al., 2005). However, there is neurochemical evidence to suggest that 5-HT₄ receptor ligands can modulate dopamine function. Agonists of the 5-HT₄ receptor stimulate dopamine release in the dorsal striatum and this effect is blocked by 5-HT₄ receptor antagonists. However, these antagonists do not alter dopamine levels in their own right suggesting that the modulatory role of 5-HT acting via 5-HT₄ receptors, on nigrostriatal dopamine is not a tonic role (Bonhomme et al., 1995; De Deurwaerdere et al., 1997; Lucas et al., 2001). In contrast to the nigrostriatal dopamine pathway it appears that 5-HT₄ receptors do not influence the functioning of the mesocorticolimbic dopamine systems (Lucas et al., 2001).

Relatively little behavioural work has been conducted using 5-HT₄ receptor ligands especially in regard to drug abuse. One study found that the 5-HT₄ receptor antagonist GR113808 reduced alcohol consumption in alcohol preferring rats

(Panocka et al., 1995). A second study reported attenuation of cocaine stimulated locomotion by the 5-HT₄ receptor antagonist SDZ 205,557, an effect that was localized to the NAc shell (McMahon and Cunningham, 1999). Other studies however have failed to find any impact of 5-HT₄ receptor antagonists on other behavioural indices of dopaminergic function. The 5-HT₄ receptor antagonist SB204070A did not alter amphetamine-stimulated locomotor activity, or amphetamine-induced rotation in unilateral 6-OHDA lesioned rats. SB204070A also failed to alter thresholds for brain stimulation reward and did not alter nicotine-induced locomotor activity (Reavill et al., 1998). On balance then the evidence suggests that 5-HT₄ receptor ligands would not have potential for modulating aspects of nicotine use and dependence related to reinforcement and reward processes.

Some evidence indicates that 5-HT₄ receptor ligands are active in animal models of anxiety and depression. In particular it has recently been shown that the 5-HT₄ receptor agonists, RS 67333 and prucalopride, exert a neurochemical and behavioural profile consistent with a rapid onset of anti-depressant action (Lucas et al., 2007). To the extent that some anti-depressant activity may be beneficial in enhancing smoking cessation, and alleviating negative mood states associated with nicotine withdrawal, further work with 5-HT₄ receptor agonists on nicotine withdrawal models may be warranted.

5-HT₆ receptors

The 5-HT₆ receptor is abundant in the major dopamine terminal areas of the dorsal striatum, NAc and frontal cortex. Lower levels of expression are found in cell body regions (Gerard et al., 1996, 1997). Several studies indicate that blockade of 5-HT₆ receptors elevates extracellular levels of dopamine in the prefrontal cortex. The 5-HT₆ receptor antagonist SB258510A potentiated the ability of amphetamine to increase dopamine levels, and the effect was more prominent in the frontal cortex than in the NAc (Frantz et al., 2002). These authors also found that SB258510A increased the locomotor stimulant effect of

amphetamine, and altered self-administration of amphetamine in a manner consistent with an increase in the reinforcing efficacy of amphetamine. Thus, following 5-HT₆ receptor blockade animals reached higher breaking points on a progressive ratio schedule of reinforcement. Interestingly, cocaine self-administration was not affected by SB258510A. In general agreement with the findings on amphetamine-induced locomotion and self-administration, another 5-HT₆ receptor antagonist MS-245 enhanced the discriminative stimulus properties of amphetamine (Pullagurla et al., 2004). This same compound also enhanced the effects of nicotine in a drug discrimination assay in rats, and increased the locomotor suppressant effects of nicotine in mice (Young et al., 2006). There is evidence then that 5-HT₆ receptor blockade, possibly via an interaction with dopaminergic function, can facilitate the behavioural effects of amphetamine and of nicotine.

In humans, a role for the 5-HT₆ receptor in smoking has been suggested based on a genetic association study (Lerer et al., 2006) reporting that Israeli women homozygous for the C276T C allele of the 5-HT₆ receptor showed significantly higher risk of smoking initiation if they had previously experienced trauma. In addition a five SNP CACCC haplotype in the 5-HT₆ receptor gene was a strong protective factor against the risk of smoking initiation. Such work suggests a possible link between 5-HT₆ receptors and smoking but much more work is needed to understand the role of 5-HT₆ receptors in smoking.

Summary and conclusions

To date the clinical effects of serotonergic-based drugs in studies of smoking cessation are largely negative. However, the majority of these studies have been conducted with SSRIs and as alluded to earlier, an SSRI-based approach is not necessarily the optimal way to manipulate 5-HT function. This is especially true given the multiplicity of receptor subtypes through which 5-HT alters neural activity. Selective pharmacological activation or inhibition of distinct subtypes is likely to

prove a more fruitful approach. For example, there is a strong preclinical rationale based on behavioural, neurochemical and electrophysiological evidence for the potential use of 5-HT_{2C} receptor agonists in smoking cessation. Given that such drugs are in advanced stages of clinical development (Smith et al., 2008) it is realistic to expect that 5-HT_{2C} receptor agonists will be assessed in smoking cessation trials within the foreseeable future. The lead indication for this drug class is obesity, and commonalities between the aetiology and treatment of eating and drug-dependence disorders are emerging (Volkow and Wise, 2005). Results with antagonists of the 5-HT_{1A} and 5-HT_{2A} receptor subtypes also offer some preclinical promise for smoking cessation but drugs acting at these receptors are yet to be evaluated in the clinic. As with other psychiatric disorders, notably schizophrenia, the treatment of smoking cessation may benefit from drugs targeting more than one 5-HT receptor subtype. As shown in this article the 5-HT_{1A}, 5-HT_{2C} and perhaps 5-HT_{2A} receptor subtypes may be the most promising for smoking cessation strategies, and multi-targeting these subtypes might present superior opportunities to single-target based approaches (Roth et al., 2004). Given the availability of excellent pharmacological tools and knockout mice for the various 5-HT receptors, interaction studies focused on behaviour relevant to nicotine use and dependence might prove fruitful.

Current evidence suggests that varenicline may represent the most efficacious smoking cessation therapy available today (Wu et al., 2006), yet relapse rates are still quite high. For example, 52 week continuous abstinence rates following varenicline treatment are reportedly 20–25% compared to 8–10% following placebo (Jorenby et al., 2006; Gonzales et al., 2007). Consequently there is a continuous need to identify and develop new pharmacotherapies for smokers who wish to quit this habit, and in this regard selective 5-HT receptor-based approaches have merit. The assessment of such drugs in animal tests which model clinically relevant and measurable aspects of smoking behaviour, such as relapse, hopefully also has translational value and may serve to de-risk subsequent clinical development.

Abbreviations

ACh	acetylcholine
CNS	central nervous system
DRN	dorsal raphe nucleus
GABA	gamma-aminobutyric acid
5-HT	5-hydroxytryptamine, serotonin
ICSS	intracranial self-stimulation
NAc	nucleus accumbens
nAChR	nicotinic acetylcholine receptor
NRT	nicotine-replacement therapy
MRN	median raphe nucleus
PTEN	phosphate and tensin homologue deleted on chromosome 10
SSRI	selective serotonin reuptake inhibitor
VTA	ventral tegmental area

References

- Andreoli, M., Tessari, M., Pilla, M., Valerio, E., Hagan, J.J. and Heidbreder, C.A. (2003) Selective antagonism at dopamine D3 receptors prevents nicotine-triggered relapse to nicotine-seeking behavior. *Neuropsychopharmacology*, 28(7): 1272–1280.
- Arnold, B., Allison, K., Ivanova, S., Paetsch, P.R., Paslawski, T. and Greenshaw, A.J. (1995) 5HT₃ receptor antagonists do not block nicotine induced hyperactivity in rats. *Psychopharmacology*, 119(2): 213–221.
- Arnt, J. (1989) Characterization of the discriminative stimulus properties induced by 5-HT₁ and 5-HT₂ agonists in rats. *Pharmacol. Toxicol.*, 64(2): 165–172.
- Ascher, J.A., Cole, J.O., Colin, J.N., Feighner, J.P., Ferris, R.M., Fibiger, H.C., Golden, R.N., Martin, P., Potter, W.Z., Richelson, E., et al. (1995) Bupropion: a review of its mechanism of antidepressant activity. *J. Clin. Psychiatry*, 56(9): 395–401.
- Auclair, A., Blanc, G., Glowinski, J. and Tassin, J.P. (2004) Role of serotonin 2A receptors in the d-amphetamine-induced release of dopamine: comparison with previous data on alpha1b-adrenergic receptors. *J. Neurochem.*, 91(2): 318–326.
- Baillie, A.J., Mattick, R.P., Hall, W. and Webster, P. (1994) Meta-analytic review of the efficacy of smoking cessation interventions. *Drug Alcohol Rev.*, 13(2): 157–170.
- Balfour, D.J. (2004) The neurobiology of tobacco dependence: a preclinical perspective on the role of the dopamine projections to the nucleus accumbens. *Nicotine Tob. Res.*, 6(6): 899–912.
- Balfour, D.J., Benwell, M.E., Birrell, C.E., Kelly, R.J. and Al-Aloul, M. (1998) Sensitization of the mesoaccumbens dopamine response to nicotine. *Pharmacol. Biochem. Behav.*, 59(4): 1021–1030.
- Balfour, D.J., Wright, A.E., Benwell, M.E. and Birrell, C.E. (2000) The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. *Behav. Brain Res.*, 113(1–2): 73–83.
- Ballidin, J., Berggren, U., Engel, J., Eriksson, M., Hard, E. and Soderpalm, B. (1994) Effect of citalopram on alcohol intake in heavy drinkers. *Alcohol Clin. Exp. Res.*, 18(5): 1133–1136.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Batman, A.M., Munzar, P. and Beardsley, P.M. (2005) Attenuation of nicotine's discriminative stimulus effects in rats and its locomotor activity effects in mice by serotonergic 5-HT_{2A/2C} receptor agonists. *Psychopharmacology*, 179(2): 393–401.
- Biberman, R., Neumann, R., Katzir, I. and Gerber, Y. (2003) A randomized controlled trial of oral selegiline plus nicotine skin patch compared with placebo plus nicotine skin patch for smoking cessation. *Addiction*, 98(10): 1403–1407.
- Blier, P., de Montigny, C. and Chaput, Y. (1990) A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J. Clin. Psychiatry*, 51(Suppl.): 14–20.
- Blondal, T., Gudmundsson, L.J., Tomasson, K., Jonsdottir, D., Hilmarsdottir, H., Kristjansson, F., Nilsson, F. and Bjornsdottir, U.S. (1999) The effects of fluoxetine combined with nicotine inhalers in smoking cessation — a randomized trial. *Addiction*, 94(7): 1007–1015.
- Boess, F.G., De Vry, J., Erb, C., Flessner, T., Hendrix, M., Luithle, J., Methfessel, C., Riedl, B., Schnizler, K., van der Staay, F.J., van Kampen, M., Wiese, W.B. and Koenig, G. (2007) The novel alpha7 nicotinic acetylcholine receptor agonist *N*-[({3R}-1-azabicyclo[2.2.2]oct-3-yl)-7-[2-(methoxy)-phenyl]-1-benzofuran-2-carboxamide improves working and recognition memory in rodents. *J. Pharmacol. Exp. Ther.*, 321(2): 716–725.
- Bonhomme, N., De Deurwaerdere, P., Le Moal, M. and Spampinato, U. (1995) Evidence for 5-HT₄ receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology*, 34(3): 269–279.
- Breese, C.R., Marks, M.J., Logel, J., Adams, C.E., Sullivan, B., Collins, A.C. and Leonard, S. (1997) Effect of smoking history on [3H]nicotine binding in human postmortem brain. *J. Pharmacol. Exp. Ther.*, 282(1): 7–13.
- Breiting, H.G., Geetha, N. and Hess, G.P. (2001) Inhibition of the serotonin 5-HT₃ receptor by nicotine, cocaine, and fluoxetine investigated by rapid chemical kinetic techniques. *Biochemistry*, 40(28): 8419–8429.
- Brioni, J.D., Kim, D.J. and O'Neill, A.B. (1996) Nicotine cue: lack of effect of the alpha 7 nicotinic receptor antagonist methyllycaconitine. *Eur. J. Pharmacol.*, 301(1–3): 1–5.
- Bruijnzeel, A.W. and Markou, A. (2004) Adaptations in cholinergic transmission in the ventral tegmental area

- associated with the affective signs of nicotine withdrawal in rats. *Neuropharmacology*, 47(4): 572–579.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Bubar, M.J., Seitz, P.K., Thomas, M.L. and Cunningham, K.A. (2005) Validation of a selective serotonin 5-HT_{2C} receptor antibody for utilization in fluorescence immunohistochemistry studies. *Brain Res.*, 1063(2): 105–113.
- Bubser, M., Backstrom, J.R., Sanders-Bush, E., Roth, B.L. and Deutch, A.Y. (2001) Distribution of serotonin 5-HT_{2A} receptors in afferents of the rat striatum. *Synapse*, 39(4): 297–304.
- Carboni, E., Acquas, E., Frau, R. and Di Chiara, G. (1989) Differential inhibitory effects of a 5-HT₃ antagonist on drug-induced stimulation of dopamine release. *Eur. J. Pharmacol.*, 164(3): 515–519.
- Carboni, E., Acquas, E., Leone, P., Perezani, L. and Di Chiara, G. (1988) 5-HT₃ receptor antagonists block morphine- and nicotine-induced place-preference conditioning. *Eur. J. Pharmacol.*, 151(1): 159–160.
- Chaudhri, N., Caggiula, A.R., Donny, E.C., Palmatier, M.I., Liu, X. and Sved, A.F. (2006) Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology*, 184(3–4): 353–366.
- Chick, J., Aschauer, H. and Hornik, K. (2004) Efficacy of fluvoxamine in preventing relapse in alcohol dependence: a one-year, double-blind, placebo-controlled multicentre study with analysis by typology. *Drug Alcohol Depend.*, 74(1): 61–70.
- Cinciripini, P.M., Lapitsky, L., Seay, S., Wallfisch, A., Meyer, W.J., III and van Vunakis, H. (1995) A placebo-controlled evaluation of the effects of buspirone on smoking cessation: differences between high- and low-anxiety smokers. *J. Clin. Psychopharmacol.*, 15(3): 182–191.
- Clarke, P.B. and Kumar, R. (1983) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. *Br. J. Pharmacol.*, 78(2): 329–337.
- Cohen, C., Bergis, O.E., Galli, F., Lochead, A.W., Jegham, S., Biton, B., Leonardon, J., Avenet, P., Sgard, F., Besnard, F., Graham, D., Coste, A., Oblin, A., Curet, O., Voltz, C., Gardes, A., Caille, D., Perrault, G., George, P., Soubrie, P. and Scatton, B. (2003) SSR591813, a novel selective and partial $\alpha_4\beta_2$ nicotinic receptor agonist with potential as an aid to smoking cessation. *J. Pharmacol. Exp. Ther.*, 306(1): 407–420.
- Cohen, C., Perrault, G., Griebel, G. and Soubrie, P. (2005) Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB₁) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology*, 30(1): 145–155.
- Cook, J.W., Spring, B., McChargue, D.E., Borrelli, B., Hitsman, B., Niaura, A., Keuthen, N.J. and Kristeller, J. (2004) Influence of fluoxetine on positive and negative affect in a clinic-based smoking cessation trial. *Psychopharmacology*, 173(1–2): 153–159.
- Cornea-Hebert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J. Comp Neurol.*, 409(2): 187–209.
- Cornelius, J.R., Perkins, K.A., Salloum, I.M., Thase, M.E. and Moss, H.B. (1999) Fluoxetine versus placebo to decrease the smoking of depressed alcoholic patients. *J. Clin. Psychopharmacol.*, 19(2): 183–184.
- Corrigall, W.A. and Coen, K.M. (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. *Psychopharmacology*, 99(4): 473–478.
- Corrigall, W.A. and Coen, K.M. (1994) Nicotine self-administration and locomotor activity are not modified by the 5-HT₃ antagonists ICS 205-930 and MDL 72222. *Pharmacol. Biochem. Behav.*, 49(1): 67–71.
- Corrigall, W.A., Coen, K.M. and Adamson, K.L. (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res.*, 653(1–2): 278–284.
- Costall, B., Domeney, A.M., Naylor, R.J. and Tyers, M.B. (1987) Effects of the 5-HT₃ receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. *Br. J. Pharmacol.*, 92(4): 881–894.
- Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Oakley, N.R., Onaivi, E.S. and Tyers, M.B. (1989) The effects of ondansetron (GR38032F) in rats and mice treated subchronically with diazepam. *Pharmacol. Biochem. Behav.*, 34(4): 769–778.
- Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Onaivi, E.S. and Tyers, M.B. (1990) Ondansetron inhibits a behavioural consequence of withdrawing from drugs of abuse. *Pharmacol. Biochem. Behav.*, 36(2): 339–344.
- Covey, L.S., Glassman, A.H., Stetner, F., Rivelli, S. and Stage, K. (2002) A randomized trial of sertraline as a cessation aid for smokers with a history of major depression. *Am. J. Psychiatry*, 159(10): 1731–1737.
- Cryan, J.F., Gasparini, F., van Hecke, G. and Markou, A. (2003) Non-nicotinic neuropharmacological strategies for nicotine dependence: beyond bupropion. *Drug Discov. Today*, 8(22): 1025–1034.
- Cryan, J.F. and Lucki, I. (2000) Antidepressant-like behavioral effects mediated by 5-hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295(3): 1120–1126.
- Davies, P.A., Pistis, M., Hanna, M.C., Peters, J.A., Lambert, J.J., Hales, T.G. and Kirkness, E.F. (1999) The 5-HT_{3B} subunit is a major determinant of serotonin-receptor function. *Nature*, 397(6717): 359–363.
- De Deurwaerdere, P., L'Hirondel, M., Bonhomme, N., Lucas, G., Cheramy, A. and Spampinato, U. (1997) Serotonin stimulation of 5-HT₄ receptors indirectly enhances in vivo dopamine release in the rat striatum. *J. Neurochem.*, 68(1): 195–203.
- de Montigny, C., Chaput, Y. and Blier, P. (1990) Modification of serotonergic neuron properties by long-term treatment with serotonin reuptake blockers. *J. Clin. Psychiatry*, 51(Suppl. B): 4–8.

- Deakin, J.F. (1998) The role of serotonin in panic, anxiety and depression. *Int. Clin. Psychopharmacol.*, 13(Suppl. 4): S1–S5.
- Di Matteo, V., Cacchio, M., Di Giulio, C. and Esposito, E. (2002) Role of serotonin_{2C} receptors in the control of brain dopaminergic function. *Pharmacol. Biochem. Behav.*, 71(4): 727–734.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38(8): 1195–1205.
- Di Matteo, V., Pierucci, M. and Esposito, E. (2004) Selective stimulation of serotonin_{2C} receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. *J. Neurochem.*, 89(2): 418–429.
- DiFranza, J.R. and Wellman, R.J. (2007) Sensitization to nicotine: how the animal literature might inform future human research. *Nicotine Tob. Res.*, 9(1): 9–20.
- Doherty, M.D. and Pickel, V.M. (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res.*, 864(2): 176–185.
- Donny, E.C., Caggiula, A.R., Knopf, S. and Brown, C. (1995) Nicotine self-administration in rats. *Psychopharmacology*, 122(4): 390–394.
- Drisdell, R.C., Sharp, D., Henderson, T., Hales, T.G. and Green, W.N. (2008) High affinity binding of epibatidine to serotonin type 3 receptors. *J. Biol. Chem.*, 288(15): 9659–9665.
- Edwards, J.G. and Anderson, I. (1999) Systematic review and guide to selection of selective serotonin reuptake inhibitors. *Drugs*, 57(4): 507–533.
- Epping-Jordan, M.P., Watkins, S.S., Koob, G.F. and Markou, A. (1998) Dramatic decreases in brain reward function during nicotine withdrawal. *Nature*, 393(6680): 76–79.
- Epstein, D.H., Preston, K.L., Stewart, J. and Shaham, Y. (2006) Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology*, 189(1): 1–16.
- Fils-Aime, M.L., Eckardt, M.J., George, D.T., Brown, G.L., Mefford, I. and Linnoila, M. (1996) Early-onset alcoholics have lower cerebrospinal fluid 5-hydroxyindoleacetic acid levels than late-onset alcoholics. *Arch. Gen. Psychiatry*, 53(3): 211–216.
- Fletcher, P.J. (1991) Dopamine receptor blockade in nucleus accumbens or caudate nucleus differentially affects feeding induced by 8-OH-DPAT injected into dorsal or median raphe. *Brain Res.*, 552(2): 181–189.
- Fletcher, P.J., Grottick, A.J. and Higgins, G.A. (2002) Differential effects of the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27(4): 576–586.
- Fletcher, P.J., Ming, Z.H. and Higgins, G.A. (1993) Conditioned place preference induced by microinjection of 8-OH-DPAT into the dorsal or median raphe nucleus. *Psychopharmacology*, 113(1): 31–36.
- Fletcher, P.J., Rizos, Z., Sinyard, J., Tampakeras, M. and Higgins, G.A. (2008) The 5-HT_{2C} receptor agonist Ro60-0175 reduces cocaine self-administration and reinstatement induced by the stressor yohimbine, and contextual cues. *Neuropsychopharmacology*, 33(6): 1402–1412.
- Fletcher, P.J., Tampakeras, M., Sinyard, J. and Higgins, G.A. (2007) Opposing effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology*, 195(2): 223–234.
- Flores, C.M., Rogers, S.W., Pabreza, L.A., Wolfe, B.B. and Kellar, K.J. (1992) A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. *Mol. Pharmacol.*, 41(1): 31–37.
- Frantz, K.J., Hansson, K.J., Stouffer, D.G. and Parsons, L.H. (2002) 5-HT₆ receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. *Neuropharmacology*, 42(2): 170–180.
- Frishman, W.H., Mitta, W., Kupersmith, A. and Ky, T. (2006) Nicotine and non-nicotine smoking cessation pharmacotherapies. *Cardiol. Rev.*, 14(2): 57–73.
- Fudala, P.J., Teoh, K.W. and Iwamoto, E.T. (1985) Pharmacologic characterization of nicotine-induced conditioned place preference. *Pharmacol. Biochem. Behav.*, 22(2): 237–241.
- Fulton, B. and Brogden, R. (1997) Buspirone: an updated review of its clinical pharmacology and therapeutic applications. *CNS Drugs*, 7: 68–88.
- Gawin, F., Compton, M. and Byck, R. (1989) Buspirone reduces smoking. *Arch. Gen. Psychiatry*, 46(3): 288–289.
- George, T.P. and O'Malley, S.S. (2004) Current pharmacological treatments for nicotine dependence. *Trends Pharmacol. Sci.*, 25(1): 42–48.
- Gerard, C., el Mestikawy, S., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23(3): 164–173.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 746(1–2): 207–219.
- Gervais, J. and Rouillard, C. (2000) Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse*, 35(4): 281–291.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36(3): 205–221.
- Gold, L.H. and Balster, R.L. (1992) Effects of buspirone and gepirone on i.v. cocaine self-administration in rhesus monkeys. *Psychopharmacology*, 108(3): 289–294.

- Gonzales, D., Rennard, S.I., Jorenby, D.E. and Reeves, K.R. (2007) Comment: oral varenicline for smoking cessation. *Ann. Pharmacother.*, 41(4): 720–721.
- Gonzales, D., Rennard, S.I., Nides, M., Oncken, C., Azoulay, S., Billing, C.B., Watsky, E.J., Gong, J., Williams, K.E. and Reeves, K.R. (2006) Varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, vs. sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. *JAMA*, 296(1): 47–55.
- Gotti, C., Moretti, M., Gaimarri, A., Zanardi, A., Clementi, F. and Zoli, M. (2007) Heterogeneity and complexity of native brain nicotinic receptors. *Biochem. Pharmacol.*, 74(8): 1102–1111.
- Grottick, A.J., Corrigan, W.A. and Higgins, G.A. (2001) Activation of 5-HT_{2C} receptors reduces the locomotor and rewarding effects of nicotine. *Psychopharmacology*, 157(3): 292–298.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000a) Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295(3): 1183–1191.
- Grottick, A.J., Trube, G., Corrigan, W.A., Huwyler, J., Malherbe, P., Wyler, R. and Higgins, G.A. (2000b) Evidence that nicotinic $\alpha 7$ receptors are not involved in the hyperlocomotor and rewarding effects of nicotine. *J. Pharmacol. Exp. Ther.*, 294(3): 1112–1119.
- Grottick, A.J., Wyler, R. and Higgins, G.A. (2000c) The $\alpha 4\beta 2$ agonist SIB 1765F, but not the $\alpha 7$ agonist AR-R 17779, cross-sensitizes to the psychostimulant effects of nicotine. *Psychopharmacology*, 150(2): 233–236.
- Gurley, D.A. and Lanthorn, T.H. (1998) Nicotinic agonists competitively antagonize serotonin at mouse 5-HT₃ receptors expressed in *Xenopus* oocytes. *Neurosci. Lett.*, 247(2–3): 107–110.
- Hagan, R.M., Jones, B.J., Jordan, C.C. and Tyers, M.B. (1990) Effect of 5-HT₃ receptor antagonists on responses to selective activation of mesolimbic dopaminergic pathways in the rat. *Br. J. Pharmacol.*, 99(2): 227–232.
- Harrison, A.A., Liem, Y.T. and Markou, A. (2001) Fluoxetine combined with a serotonin-1A receptor antagonist reversed reward deficits observed during nicotine and amphetamine withdrawal in rats. *Neuropsychopharmacology*, 25(1): 55–71.
- Hatsukami, D.K., Jensen, J., Brauer, L.H., Mooney, M., Schulte, S., Sofuoglu, M. and Pentel, P.R. (2003) Lack of effect of 5HT₃ antagonist in mediating subjective and behavioral responses to cotinine. *Pharmacol. Biochem. Behav.*, 75(1): 1–7.
- Helton, D.R., Modlin, D.L., Tizzano, J.P. and Rasmussen, K. (1993) Nicotine withdrawal: a behavioral assessment using schedule controlled responding, locomotor activity, and sensorimotor reactivity. *Psychopharmacology*, 113(2): 205–210.
- Hemeryck, A. and Belpaire, F.M. (2002) Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug–drug interactions: an update. *Curr. Drug Metab.*, 3(1): 13–37.
- Higgins, G.A. and Fletcher, P.J. (2003) Serotonin and drug reward: focus on 5-HT_{2C} receptors. *Eur. J. Pharmacol.*, 480(1–3): 151–162.
- Hilleman, D.E., Mohiuddin, S.M., Del Core, M.G. and Sketch, M.H., Sr. (1992) Effect of buspirone on withdrawal symptoms associated with smoking cessation. *Arch. Intern. Med.*, 152(2): 350–352.
- Hitsman, B., Pingitore, R., Spring, B., Mahableshwarkar, A., Mizes, J.S., Segraves, K.A., Kristeller, J.L. and Xu, W. (1999) Antidepressant pharmacotherapy helps some cigarette smokers more than others. *J. Consult. Clin. Psychol.*, 67(4): 547–554.
- Hjorth, S. and Magnusson, T. (1988) The 5-HT 1A receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 338(5): 463–471.
- Homberg, J.R., Arends, B., Wardeh, G., Raaso, H.S., Schoffelmeer, A.N. and de Vries, T.J. (2004) Individual differences in the effects of serotonergic anxiolytic drugs on the motivation to self-administer cocaine. *Neuroscience*, 128(1): 121–130.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Hughes, J.R., Gust, S.W., Skoog, K., Keenan, R.M. and Fenwick, J.W. (1991) Symptoms of tobacco withdrawal: a replication and extension. *Arch. Gen. Psychiatry*, 48(1): 52–59.
- Hughes, J.R., Stead, L.F. and Lancaster, T. (2007) Antidepressants for smoking cessation. *Cochrane Database Syst. Rev.*, (1): p. CD000031.
- Hurt, R.D., Sachs, D.P., Glover, E.D., Offord, K.P., Johnston, J.A., Dale, L.C., Khayrallah, M.A., Schroeder, D.R., Glover, P.N., Sullivan, C.R., Croghan, I.T. and Sullivan, P.M. (1997) A comparison of sustained-release bupropion and placebo for smoking cessation. *N. Engl. J. Med.*, 337(17): 1195–1202.
- Hutson, P.H., Dourish, C.T. and Curzon, G. (1986) Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors. *Eur. J. Pharmacol.*, 129(3): 347–352.
- Huston-Lyons, D. and Kornetsky, C. (1992) Effects of nicotine on the threshold for rewarding brain stimulation in rats. *Pharmacol. Biochem. Behav.*, 41(4): 755–759.
- Ichikawa, J. and Meltzer, H.Y. (1995) DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. *Brain Res.*, 698(1–2): 204–208.
- Ivanova, S. and Greenshaw, A.J. (1997) Nicotine-induced decreases in VTA electrical self-stimulation thresholds: blockade by haloperidol and mecamylamine but not scopolamine or ondansetron. *Psychopharmacology*, 134(2): 187–192.
- Jackson, K.J., Martin, B.R., Changeux, J.P. and Damaj, M.I. (2008) Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *J. Pharmacol. Exp. Ther.*, 325(1): 302–312.
- Ji, S.P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R. and Zhang, X. (2006) Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat. Med.*, 12(3): 324–329.

- Johnson, S.W., Mercuri, N.B. and North, R.A. (1992) 5-hydroxytryptamine_{1B} receptors block the GABAB synaptic potential in rat dopamine neurons. *J. Neurosci.*, 12(5): 2000–2006.
- Jorenby, D.E., Hays, J.T., Rigotti, N.A., Azoulay, S., Watsky, E.J., Williams, K.E., Billing, C.B., Gong, J. and Reeves, K.R. (2006) Efficacy of varenicline, an $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, vs. placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. *JAMA*, 296(1): 56–63.
- Kenford, S.L., Fiore, M.C., Jorenby, D.E., Smith, S.S., Wetter, D. and Baker, T.B. (1994) Predicting smoking cessation: who will quit with and without the nicotine patch. *JAMA*, 271(8): 589–594.
- Killen, J.D., Fortmann, S.P., Schatzberg, A.F., Hayward, C., Sussman, L., Rothman, M., Strausberg, L. and Varady, A. (2000) Nicotine patch and paroxetine for smoking cessation. *J. Consult Clin. Psychol.*, 68(5): 883–889.
- Kranzler, H.R., Burleson, J.A., Brown, J. and Babor, T.F. (1996) Fluoxetine treatment seems to reduce the beneficial effects of cognitive-behavioral therapy in type B alcoholics. *Alcohol Clin. Exp. Res.*, 20(9): 1534–1541.
- Lavolette, S.R. and van der Kooy, D. (2001) GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. *Eur. J. Neurosci.*, 13(5): 1009–1015.
- Lerer, E., Kanyas, K., Karni, O., Ebstein, R.P. and Lerer, B. (2006) Why do young women smoke? II. Role of traumatic life experience, psychological characteristics and serotonergic genes. *Mol. Psychiatry*, 11(8): 771–781.
- Lerman, C., Lesage, M.G., Perkins, K.A., O'Malley, S.S., Siegel, S.J., Benowitz, N.L. and Corrigan, W.A. (2007) Translational research in medication development for nicotine dependence. *Nat. Rev. Drug Discov.*, 6(9): 746–762.
- Lucas, G., Di Matteo, V., De Deurwaerdere, P., Porras, G., Martin-Ruiz, R., Artigas, F., Esposito, E. and Spampinato, U. (2001) Neurochemical and electrophysiological evidence that 5-HT₄ receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal, dopaminergic function. *Eur. J. Neurosci.*, 13(5): 889–898.
- Lucas, G., Rymar, V.V., Du, J., Mnie-Filali, O., Bisgaard, C., Manta, S., Lambas-Senas, L., Wiborg, O., Haddjeri, N., Pineyro, G., Sadikot, A.F. and Debonnel, G. (2007) Serotonin(4) (5-HT₄) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron*, 55(5): 712–725.
- Mandrioli, R., Forti, G.C. and Raggi, M.A. (2006) Fluoxetine metabolism and pharmacological interactions: the role of cytochrome p450. *Curr. Drug Metab.*, 7(2): 127–133.
- Mansvelder, H.D. and McGehee, D.S. (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron*, 27(2): 349–357.
- Mansvelder, H.D. and McGehee, D.S. (2002) Cellular and synaptic mechanisms of nicotine addiction. *J. Neurobiol.*, 53(4): 606–617.
- Marcinkiewicz, M., Verge, D., Gozlan, H., Pichat, L. and Hamon, M. (1984) Autoradiographic evidence for the heterogeneity of 5-HT₁ sites in the rat brain. *Brain Res.*, 291(1): 159–163.
- Markou, A. and Paterson, N.E. (2001) The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. *Nicotine Tob. Res.*, 3(4): 361–373.
- Martin, J.R., Bos, M., Jenck, F., Moreau, J., Mutel, V., Sleight, A.J., Wichmann, J., Andrews, J.S., Berendsen, H.H., Broekkamp, C.L., Ruigt, G.S., Kohler, C. and Delft, A.M. (1998) 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.*, 286(2): 913–924.
- Marubio, L.M., Gardier, A.M., Durier, S., David, D., Klink, R., Arroyo-Jimenez, M.M., McIntosh, J.M., Rossi, F., Chantiaux, N., Zoli, M. and Changeux, J.P. (2003) Effects of nicotine in the dopaminergic system of mice lacking the $\alpha 4$ subunit of neuronal nicotinic acetylcholine receptors. *Eur. J. Neurosci.*, 17(7): 1329–1337.
- Maskos, U., Molles, B.E., Pons, S., Besson, M., Guiard, B.P., Guilloux, J.P., Evrard, A., Cazala, P., Cormier, A., Mameli-Engvall, M., Dufour, N., Cloez-Tayarani, I., Bemelmans, A.P., Mallet, J., Gardier, A.M., David, V., Faure, P., Granon, S. and Changeux, J.P. (2005) Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature*, 436(7047): 103–107.
- Mayhew, K.P., Flay, B.R. and Mott, J.A. (2000) Stages in the development of adolescent smoking. *Drug Alcohol Depend.*, 59(Suppl. 1): S61–S81.
- McMahon, L.R. and Cunningham, K.A. (1999) Antagonism of 5-hydroxytryptamine(4) receptors attenuates hyperactivity induced by cocaine: putative role for 5-hydroxytryptamine(4) receptors in the nucleus accumbens shell. *J. Pharmacol. Exp. Ther.*, 291(1): 300–307.
- McMahon, L.R. and Cunningham, K.A. (2001) Antagonism of 5-hydroxytryptamine_{2A} receptors attenuates the behavioral effects of cocaine in rats. *J. Pharmacol. Exp. Ther.*, 297(1): 357–363.
- McMahon, L.R., Filip, M. and Cunningham, K.A. (2001) Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)_{2A} and 5-HT_{2C} receptors. *J. Neurosci.*, 21(19): 7781–7787.
- Mengod, G., Pompeiano, M., Martinez-Mir, M.I. and Palacios, J.M. (1990) Localization of the mRNA for the 5-HT₂ receptor by in situ hybridization histochemistry: correlation with the distribution of receptor sites. *Brain Res.*, 524(1): 139–143.
- Moreau, J.L., Bos, M., Jenck, F., Martin, J.R., Mortas, P. and Wichmann, J. (1996) 5HT_{2C} receptor agonists exhibit antidepressant-like properties in the anhedonia model of depression in rats. *Eur. Neuropsychopharmacol.*, 6(3): 169–175.
- Mosner, A., Kuhlman, G., Roehm, C. and Vogel, W.H. (1997) Serotonergic receptors modify the voluntary intake of alcohol and morphine but not of cocaine and nicotine by rats. *Pharmacology*, 54(4): 186–192.
- Muller, C.P. and Carey, R.J. (2006) Intracellular 5-HT_{2C}-receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol. Sci.*, 27(9): 455–458.

- Naranjo, C.A., Bremner, K.E. and Lanctot, K.L. (1995) Effects of citalopram and a brief psycho-social intervention on alcohol intake, dependence and problems. *Addiction*, 90(1): 87–99.
- Niaura, R., Spring, B., Borrelli, B., Hedeker, D., Goldstein, M.G., Keuthen, N., DePue, J., Kristeller, J., Ockene, J., Prochazka, A., Chiles, J.A. and Abrams, D.B. (2002) Multicenter trial of fluoxetine as an adjunct to behavioral smoking cessation treatment. *J. Consult Clin. Psychol.*, 70(4): 887–896.
- Nichols, D.E. (2004) Hallucinogens. *Pharmacol. Ther.*, 101(2): 131–181.
- Niesler, B., Walstab, J., Combrink, S., Moller, D., Kapeller, J., Rietdorf, J., Bonisch, H., Gothert, M., Rappold, G. and Bruss, M. (2007) Characterization of the novel human serotonin receptor subunits 5-HT3C, 5-HT3D, and 5-HT3E. *Mol. Pharmacol.*, 72(1): 8–17.
- Nocjar, C., Roth, B.L. and Pehek, E.A. (2002) Localization of 5-HT2A receptors on dopamine cells in subnuclei of the midbrain A10 cell group. *Neuroscience*, 111(1): 163–176.
- Olausson, P., Akesson, P., Engel, J.A. and Soderpalm, B. (2001) Effects of 5-HT1A and 5-HT2 receptor agonists on the behavioral and neurochemical consequences of repeated nicotine treatment. *Eur. J. Pharmacol.*, 420(1): 45–54.
- O'Neill, M.F., Heron-Maxwell, C.L. and Shaw, G. (1999) 5-HT2 receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. *Pharmacol. Biochem. Behav.*, 63(2): 237–243.
- Panocka, I., Ciccocioppo, R., Polidori, C., Pompei, P. and Massi, M. (1995) The 5-HT4 receptor antagonist, GR113808, reduces ethanol intake in alcohol-preferring rats. *Pharmacol. Biochem. Behav.*, 52(2): 255–259.
- Patel, S., Roberts, J., Moorman, J. and Reavill, C. (1995) Localization of serotonin-4 receptors in the striatonigral pathway in rat brain. *Neuroscience*, 69(4): 1159–1167.
- Pazos, A., Cortes, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.*, 346(2): 231–249.
- Pazos, A., Hoyer, D. and Palacios, J.M. (1984) The binding of serotonergic ligands to the porcine choroid plexus: characterization of a new type of serotonin recognition site. *Eur. J. Pharmacol.*, 106(3): 539–546.
- Pehek, E.A., McFarlane, H.G., Maguschak, K., Price, B. and Pluto, C.P. (2001) M100,907, a selective 5-HT2A antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res.*, 888(1): 51–59.
- Peltier, R. and Schenk, S. (1993) Effects of serotonergic manipulations on cocaine self-administration in rats. *Psychopharmacology*, 110(4): 390–394.
- Perkins, K.A., Gerlach, D., Broge, M., Sanders, M., Grobe, J., Fonte, C., Cherry, C., Wilson, A. and Jacob, R. (2001) Quitting cigarette smoking produces minimal loss of chronic tolerance to nicotine. *Psychopharmacology*, 158(1): 7–17.
- Perry, D.C., Davila-Garcia, M.I., Stockmeier, C.A. and Kellar, K.J. (1999) Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. *J. Pharmacol. Exp. Ther.*, 289(3): 1545–1552.
- Picciotto, M.R., Zoli, M. and Changeux, J.P. (1999) Use of knock-out mice to determine the molecular basis for the actions of nicotine. *Nicotine Tob. Res.*, 1(Suppl. 2): S121–S125.
- Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L.M., Pich, E.M., Fuxe, K. and Changeux, J.P. (1998) Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature*, 391(6663): 173–177.
- Pierucci, M., Di Matteo, V. and Esposito, E. (2004) Stimulation of serotonin2C receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.*, 309(1): 109–118.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12(2): 440–453.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res. Mol. Brain Res.*, 23(1–2): 163–178.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E. and Spampinato, U. (2002) 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 311–324.
- Pratt, G.D., Bowery, N.G., Kilpatrick, G.J., Leslie, R.A., Barnes, N.M., Naylor, R.J., Jones, B.J., Nelson, D.R., Palacios, J.M., Slater, P., et al. (1990) Consensus meeting agrees distribution of 5-HT3 receptors in mammalian hindbrain. *Trends Pharmacol. Sci.*, 11(4): 135–137.
- Prisco, S., Pagannone, S. and Esposito, E. (1994) Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. *J. Pharmacol. Exp. Ther.*, 271(1): 83–90.
- Pullagurla, M., Bondareva, T., Young, R. and Glennon, R.A. (2004) Modulation of the stimulus effects of (+)amphetamine by the 5-HT6 antagonist MS-245. *Pharmacol. Biochem. Behav.*, 78(2): 263–268.
- Quarta, D., Naylor, C.G. and Stolerman, I.P. (2007) The serotonin 2C receptor agonist Ro-60-0175 attenuates effects of nicotine in the five-choice serial reaction time task and in drug discrimination. *Psychopharmacology*, 193(3): 391–402.
- Rasmussen, K., Calligaro, D.O., Czachura, J.F., Dreshfield-Ahmad, L.J., Evans, D.C., Hemrick-Luecke, S.K., Kallman, M.J., Kendrick, W.T., Leander, J.D., Nelson, D.L., Overshiner, C.D., Wainwright, D.B., Wolff, M.C., Wong, D.T., Branchek, T.A., Zgombick, J.M. and Xu, Y.C. (2000) The novel 5-hydroxytryptamine1A antagonist LY426965: effects on nicotine withdrawal and interactions with fluoxetine. *J. Pharmacol. Exp. Ther.*, 294(2): 688–700.
- Rasmussen, K., Kallman, M.J. and Helton, D.R. (1997) Serotonin-1A antagonists attenuate the effects of nicotine withdrawal on the auditory startle response. *Synapse*, 27(2): 145–152.

- Reavill, C., Hatcher, J.P., Lewis, V.A., Sanger, G.J. and Hagan, J. (1998) 5-HT₄ receptor antagonism does not affect motor and reward mechanisms in the rat. *Eur. J. Pharmacol.*, 357(2–3): 115–120.
- Reavill, C. and Stolerman, I.P. (1990) Locomotor activity in rats after administration of nicotinic agonists intracerebrally. *Br. J. Pharmacol.*, 99(2): 273–278.
- Risinger, F.O. and Oakes, R.A. (1995) Nicotine-induced conditioned place preference and conditioned place aversion in mice. *Pharmacol. Biochem. Behav.*, 51(2–3): 457–461.
- Robinson, M.D., Pettice, Y.L., Smith, W.A., Cederstrom, E.A., Sutherland, D.E. and Davis, H. (1992) Buspirone effect on tobacco withdrawal symptoms: a randomized placebo-controlled trial. *J. Am. Board Fam. Pract.*, 5(1): 1–9.
- Robinson, M.D., Smith, W.A., Cederstrom, E.A. and Sutherland, D.E. (1991) Buspirone effect on tobacco withdrawal symptoms: a pilot study. *J. Am. Board Fam. Pract.*, 4(2): 89–94.
- Robinson, S.E., James, J.R., Lapp, L.N., Vann, R.E., Gross, D.F., Philibin, S.D. and Rosecrans, J.A. (2006) Evidence of cellular nicotinic receptor desensitization in rats exhibiting nicotine-induced acute tolerance. *Psychopharmacology*, 184(3–4): 306–313.
- Robinson, T.E. and Berridge, K.C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Brain Res. Rev.*, 18(3): 247–291.
- Rollema, H., Chambers, L.K., Coe, J.W., Glowa, J., Hurst, R.S., Lebel, L.A., Lu, Y., Mansbach, R.S., Mather, R.J., Rovetti, C.C., Sands, S.B., Schaeffer, E., Schulz, D.W., Tingley, F.D., III and Williams, K.E. (2007a) Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology*, 52(3): 985–994.
- Rollema, H., Coe, J.W., Chambers, L.K., Hurst, R.S., Stahl, S.M. and Williams, K.E. (2007b) Rationale, pharmacology and clinical efficacy of partial agonists of alpha4beta2nACh receptors for smoking cessation. *Trends Pharmacol. Sci.*, 28(7): 316–325.
- Rosenzweig-Lipson, S., Sabb, A., Stack, G., Mitchell, P., Lucki, I., Malberg, J.E., Grauer, S., Brennan, J., Cryan, J.F., Sukoff Rizzo, S.J., Dunlop, J., Barrett, J.E. and Marquis, K.L. (2007) Antidepressant-like effects of the novel, selective, 5-HT_{2C} receptor agonist WAY-163909 in rodents. *Psychopharmacology*, 192(2): 159–170.
- Ross, J.T., Corrigan, W.A., Heidbreder, C.A. and LeSage, M.G. (2007) Effects of the selective dopamine D₃ receptor antagonist SB-277011A on the reinforcing effects of nicotine as measured by a progressive-ratio schedule in rats. *Eur. J. Pharmacol.*, 559(2–3): 173–179.
- Roth, B.L., Hanizavareh, S.M. and Blum, A.E. (2004) Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology*, 174(1): 17–24.
- Schechter, M.D. and Meehan, S.M. (1992) Further evidence for the mechanisms that may mediate nicotine discrimination. *Pharmacol. Biochem. Behav.*, 41(4): 807–812.
- Schenk, S. (2000) Effects of the serotonin 5-HT₂ antagonist, ritanserin, and the serotonin 5-HT_{1A} antagonist, WAY 100635, on cocaine-seeking in rats. *Pharmacol. Biochem. Behav.*, 67(2): 363–369.
- Schneider, N.G., Olmstead, R.E., Steinberg, C., Sloan, K., Daims, R.M. and Brown, H.V. (1996) Efficacy of buspirone in smoking cessation: a placebo-controlled trial. *Clin. Pharmacol. Ther.*, 60(5): 568–575.
- Schnoll, R.A. and Lerman, C. (2006) Current and emerging pharmacotherapies for treating tobacco dependence. *Expert Opin. Emerg. Drugs*, 11(3): 429–444.
- Shaham, Y., Shalev, U., Lu, L., De Wit, H. and Stewart, J. (2003) The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*, 168(1–2): 3–20.
- Shiffman, S.M. and Jarvik, M.E. (1976) Smoking withdrawal symptoms in two weeks of abstinence. *Psychopharmacology*, 50(1): 35–39.
- Shoaib, M., Gommans, J., Morley, A., Stolerman, I.P., Grailhe, R. and Changeux, J.P. (2002) The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. *Neuropharmacology*, 42(4): 530–539.
- Shoaib, M., Zubarán, C. and Stolerman, I.P. (2000) Antagonism of stimulus properties of nicotine by dihydro-beta-erythroidine (DHbetaE) in rats. *Psychopharmacology*, 149(2): 140–146.
- Siu, E.C. and Tyndale, R.F. (2007) Non-nicotinic therapies for smoking cessation. *Annu. Rev. Pharmacol. Toxicol.*, 47: 541–564.
- Smith, B.M., Smith, J.M., Tsai, J.H., Schultz, J.A., Gilson, C.A., Estrada, S.A., Chen, R.R., Park, D.M., Prieto, E.B., Gallardo, C.S., Sengupta, D., Dosa, P.I., Covell, J.A., Ren, A., Webb, R.R., Beeley, N.R., Martin, M., Morgan, M., Espitia, S., Saldana, H.R., Bjenning, C., Whelan, K.T., Grottick, A.J., Menzaghi, F. and Thomsen, W.J. (2008) Discovery and structure-activity relationship of (1R)-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3-benzazepine (Lorcaserin), a selective serotonin 5-HT_{2C} receptor agonist for the treatment of obesity. *J. Med. Chem.*, 51(2): 305–313.
- Spring, B., Doran, N., Pagoto, S., McChargue, D., Cook, J.W., Bailey, K., Crayton, J. and Hedeker, D. (2007) Fluoxetine, smoking, and history of major depression: a randomized controlled trial. *J. Consult Clin. Psychol.*, 75(1): 85–94.
- Stolerman, I.P., Chamberlain, S., Bizarro, L., Fernandes, C. and Schalkwyk, L. (2004) The role of nicotinic receptor alpha 7 subunits in nicotine discrimination. *Neuropharmacology*, 46(3): 363–371.
- Stolerman, I.P., Garcha, H.S., Pratt, J.A. and Kumar, R. (1984) Role of training dose in discrimination of nicotine and related compounds by rats. *Psychopharmacology*, 84(3): 413–419.
- Stolerman, I.P. and Jarvis, M.J. (1995) The scientific case that nicotine is addictive. *Psychopharmacology*, 117(1): 2–10. discussion 14–20.
- Suzuki, T., Ise, Y., Mori, T. and Misawa, M. (1997) Attenuation of mecamylamine-precipitated nicotine-withdrawal aversion

- by the 5-HT₃ receptor antagonist ondansetron. *Life Sci.*, 61(16): PL249–PL254.
- Tapper, A.R., McKinney, S.L., Nashmi, R., Schwarz, J., Deshpande, P., Labarca, C., Whiteaker, P., Marks, M.J., Collins, A.C. and Lester, H.A. (2004) Nicotine activation of $\alpha 4^*$ receptors: sufficient for reward, tolerance, and sensitization. *Science*, 306(5698): 1029–1032.
- Tomkins, D.M., Sellers, E.M. and Fletcher, P.J. (1994) Median and dorsal raphe injections of the 5-HT_{1A} agonist, 8-OH-DPAT, and the GABA_A agonist, muscimol, increase voluntary ethanol intake in Wistar rats. *Neuropharmacology*, 33(3–4): 349–358.
- Tork, I. (1990) Anatomy of the serotonergic system. *Ann. N.Y. Acad. Sci.*, 600: 9–34. discussion 34–35
- Verge, D., Daval, G., Marcinkiewicz, M., Patey, A., el Mestikawy, S., Gozlan, H. and Hamon, M. (1986) Quantitative autoradiography of multiple 5-HT₁ receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J. Neurosci.*, 6(12): 3474–3482.
- Vilaro, M.T., Cortes, R. and Mengod, G. (2005) Serotonin 5-HT₄ receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. *J. Comp. Neurol.*, 484(4): 418–439.
- Virkkunen, M., Rawlings, R., Tokola, R., Poland, R.E., Guidotti, A., Nemeroff, C., Bissette, G., Kalogeras, K., Karonen, S.L. and Linnoila, M. (1994) CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. *Arch. Gen. Psychiatry*, 51(1): 20–27.
- Volkow, N.D. and Wise, R.A. (2005) How can drug addiction help us understand obesity? *Nat. Neurosci.*, 8(5): 555–560.
- Watkins, S.S., Epping-Jordan, M.P., Koob, G.F. and Markou, A. (1999) Blockade of nicotine self-administration with nicotinic antagonists in rats. *Pharmacol. Biochem. Behav.*, 62(4): 743–751.
- Watkins, S.S., Stinus, L., Koob, G.F. and Markou, A. (2000) Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. *J. Pharmacol. Exp. Ther.*, 292(3): 1053–1064.
- West, R. and Hajek, P. (1996) Randomised controlled trial of ondansetron in smoking cessation. *Psychopharmacology*, 126(1): 95–96.
- West, R., Hajek, P. and McNeill, A. (1991) Effect of buspirone on cigarette withdrawal symptoms and short-term abstinence rates in a smoker's clinic. *Psychopharmacology*, 104(1): 91–96.
- West, R.J., Hajek, P. and Belcher, M. (1989) Severity of withdrawal symptoms as a predictor of outcome of an attempt to quit smoking. *Psychol. Med.*, 19(4): 981–985.
- Willins, D.L., Deutch, A.Y. and Roth, B.L. (1997) Serotonin 5-HT_{2A} receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse*, 27(1): 79–82.
- Wu, P., Wilson, K., Dimoulas, P. and Mills, E.J. (2006) Effectiveness of smoking cessation therapies: a systematic review and meta-analysis. *BMC Public Health*, 6: p. 300.
- Yan, Q., Reith, M.E. and Yan, S. (2000) Enhanced accumbal dopamine release following 5-HT_{2A} receptor stimulation in rats pretreated with intermittent cocaine. *Brain Res.*, 863(1–2): 254–258.
- Young, R., Bondareva, T., Wesolowska, A., Young, S. and Glennon, R.A. (2006) Effect of the 5-HT₆ serotonin antagonist MS-245 on the actions of (–)nicotine. *Pharmacol. Biochem. Behav.*, 85(1): 170–177.
- Zacny, J.P., Apfelbaum, J.L., Lichter, J.L. and Zaragoza, J.G. (1993) Effects of 5-hydroxytryptamine₃ antagonist, ondansetron, on cigarette smoking, smoke exposure, and mood in humans. *Pharmacol. Biochem. Behav.*, 44(2): 387–391.
- Zaniewska, M., McCreary, A.C., Przegalinski, E. and Filip, M. (2007) Effects of the serotonin 5-HT_{2A} and 5-HT_{2C} receptor ligands on the discriminative stimulus effects of nicotine in rats. *Eur. J. Pharmacol.*, 571: 156–165.

CHAPTER 19

Dopamine/serotonin releasers as medications for stimulant addictions

Richard B. Rothman^{1,*}, Bruce E. Blough² and Michael H. Baumann¹

¹*Clinical Psychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, DHHS, Baltimore, MD, USA*

²*Chemistry and Life Sciences Group, Research Triangle Institute International, Research Triangle Park, NC, USA*

Abstract: The use of ‘agonist therapy’ for cocaine and methamphetamine addiction involves administration of stimulant-like medications (e.g. monoamine releasers) to reduce withdrawal symptoms and prevent relapse. A significant problem with this strategy is that many candidate medications possess abuse liability due to activation of mesolimbic dopamine (DA) neurons in the brain. One way to reduce DA-mediated abuse liability of candidate drugs might be to add in serotonin (5-HT)-releasing properties, since substantial evidence shows that 5-HT neurons provide an inhibitory influence over mesolimbic DA neurons. This chapter addresses several key issues related to the development of dual DA/5-HT releasers for the treatment of substance use disorders. First, we briefly summarize the evidence supporting a dual deficit in DA and 5-HT function during withdrawal from chronic cocaine or alcohol abuse. Second, we discuss data demonstrating that 5-HT release can dampen DA-mediated stimulant effects, and the ‘anti-stimulant’ role of 5-HT_{2C} receptors is considered. Next, the mechanisms underlying potential adverse effects of 5-HT releasers are described. Finally, we discuss recently published data with PAL-287, a novel non-amphetamine DA/5-HT-releasing agent that suppresses cocaine self-administration but lacks positive reinforcing properties. It is concluded that DA/5-HT releasers could be useful therapeutic adjuncts for the treatment of cocaine and alcohol addictions as well as for obesity, attention deficit disorder and depression.

Keywords: alcohol; amphetamine; cocaine; dopamine; serotonin; treatment; transporter

Introduction

A main goal of this chapter is to review data from our laboratory pertaining to the development of dual dopamine (DA)/serotonin (5-HT) releasers as medications for stimulant addiction and possibly alcohol addiction (Rea et al., 1998; Wojnicki et al., 1999; Baumann et al., 2000, 2001; Rothman and

Baumann, 2003; Rothman et al., 2005; Wee et al., 2005). A secondary goal is to integrate our findings with the existing literature to provide a conceptual framework for the design of new medications for addiction disorders. Within the context of this chapter, the term ‘stimulant’ refers to drugs such as cocaine and amphetamines that produce a spectrum of effects in humans, including cardiovascular stimulation, mood elevation and a decreased need for sleep. At high doses, or after longer periods of use, stimulants can cause a range of adverse effects, such as disordered thoughts and

*Corresponding author. Tel.: + (410) 550-1598;
Fax: + (410) 550-2997; E-mail: rrothman@mail.nih.gov

psychotic episodes. In laboratory animals, stimulants increase locomotor activity and are readily self-administered due to their powerful reinforcing properties. Figure 1 depicts the chemical structures of drugs mentioned in this chapter. Many of these drugs are useful medications with long histories of efficacy and safety while others are highly addictive substances associated with considerable morbidity and mortality (Musto, 1992; Das, 1993; Anonymous, 1995; Gonzalez Castro et al., 2000). In some cases, as with amphetamine itself, the same drug can be a therapeutic entity or an abused substance, depending upon the context in which the drug is administered (Arnsten, 2006; Greenhill, 2006).

Most stimulant drugs interact with monoamine neurons in the central nervous system (CNS). Neurons that synthesize, store and release monoamine transmitters — norepinephrine (NE), dopamine (DA) and serotonin (5-HT) — are widely distributed in the mammalian CNS. These neurons express specialized plasma membrane proteins that function to transport previously released transmitter molecules from the extracellular space back into the cytoplasm (Amara and Kuhar, 1993; Masson et al., 1999). It is well established that

distinct transporter proteins are expressed by each type of monoamine neuron: NE transporters (NET), DA transporters (DAT) and 5-HT transporters (SERT) are associated with NE, DA and 5-HT neurons, respectively. These proteins belong to a superfamily of Na^+/Cl^- -dependent transporters that share genetic, morphological and functional homologies (Uhl and Johnson, 1994; Torres and Amara, 2007). Under normal circumstances, the transporter-mediated uptake of monoamine transmitters is the principal mechanism for inactivation of monoamine signalling in the brain. Consequently, drugs that interact with monoamine transporters have profound effects on CNS function, and these effects can be beneficial or detrimental depending upon the dose, route and formulation of the drug administered (Amara and Sonders, 1998; Iversen, 2006).

Drugs that target transporter proteins can be divided into two classes based on their precise mechanism of action: reuptake inhibitors and substrate-type releasers (Rothman and Baumann, 2003). Reuptake inhibitors bind to transporter proteins but are not transported. These drugs elevate extracellular transmitter concentrations by blocking transporter-mediated recapture of

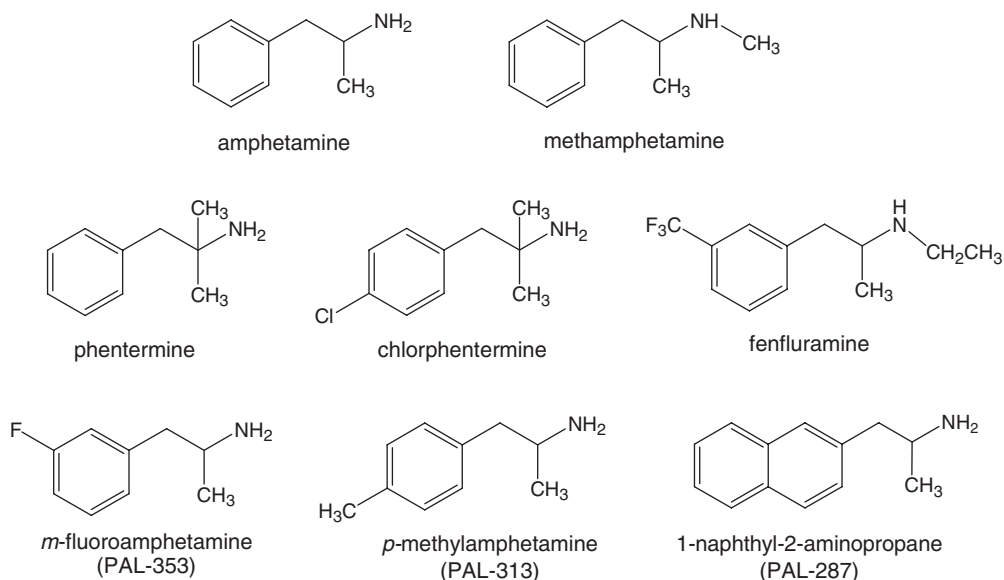


Fig. 1. Chemical structures of stimulants discussed in this chapter.

transmitter molecules from the synapse. Substrate-type releasers bind to transporter proteins and are subsequently transported into the cytoplasm of nerve terminals. Thus, transmitter ‘releasers’ are often referred to as transporter ‘substrates’. Releasers elevate extracellular transmitter concentrations by a two-pronged mechanism: (1) they promote efflux of transmitter by a process of transporter-mediated exchange and (2) they increase cytoplasmic levels of transmitter by disrupting storage of transmitters in vesicles via interactions with the vesicular monoamine transporter 2 (VMAT2) (Rudnick, 1997; Fleckenstein et al., 2007). The molecular mechanisms underlying transporter-mediated transmitter release are not completely understood, but ionic currents, oligomerization and reversal of normal transport function appear to be involved (Sitte and Freissmuth, 2003; Blakely et al., 2005; Sulzer et al., 2005). Because substrate-type releasing agents must be transported into nerve terminals to promote transmitter release, reuptake inhibitors can block the effects of releasers.

A dual deficit model of stimulant addiction

The use of stimulants such as cocaine and methamphetamine produces a ‘high’ or ‘rush’ that is likely mediated by elevations in extracellular DA levels in mesolimbic circuits (Volkow et al., 2002; Di Chiara et al., 2004), although some evidence indicates that elevations in extracellular NE may also contribute (Rothman et al., 2001; Alexander et al., 2005). Similarly, alcohol-induced increases in extracellular DA are thought to underlie the positive reinforcing effects of this commonly abused substance (Koob et al., 1998; Koob, 2003). Repeated misuse of stimulants, especially when they are self-administered via the smoked or intravenous routes, can lead to serious addiction in susceptible individuals. The chronic abuse of stimulants and alcohol, despite negative consequences, causes long-term changes in neurochemistry and brain circuitry via processes of synaptic plasticity (Volkow and Li, 2004; Hyman, 2005; Kalivas and O’Brien, 2008).

Preclinical and human research findings demonstrate that withdrawal from stimulant and alcohol abuse is associated with deficits in DA and 5-HT function. For example, rats withdrawn from chronic cocaine or alcohol administration display decreased levels of extracellular DA and 5-HT in the nucleus accumbens (Parsons et al., 1991, 1995; Rossetti et al., 1992; Weiss et al., 1996). Human brain imaging studies show that cocaine addicts have reductions in evoked DA release and a loss of DA D₂ receptors in the striatum (Volkow et al., 1997, 2002, Martinez et al., 2007). Neuroendocrine responsiveness to 5-HT releasers is diminished in rats withdrawn from repeated cocaine injections (Levy et al., 1994; Baumann et al., 1995a), and similar findings have been reported in abstinent human cocaine addicts (Haney et al., 2001; Ghitza et al., 2007). Taken together, these data suggest that a cardinal feature of withdrawal from chronic cocaine, and possibly alcohol, is decreased synaptic levels of DA and 5-HT in critical brain circuits.

Additional clinical support for the existence of 5-HT deficits in cocaine addicts is the occurrence of symptoms resembling major depression during abstinence (Dackis and Gold, 1985; Gawin and Kleber, 1986), coupled with an increased prevalence of suicidal ideation and suicide attempts (Garlow et al., 2003). The well-accepted importance of 5-HT dysfunction in mediating depression and suicide (for review, see Mann, 2003) suggests a parallel role for decreased synaptic 5-HT in cocaine and alcohol withdrawal states (Lesch, 2005). Indeed, the spectrum of symptoms often reported by patients withdrawing from stimulant or alcohol use — depressed mood, suicidal ideations, obsessive thoughts, intense craving, anhedonia, increased impulsivity and susceptibility to drug-related cues — presumably reflects long-term changes in brain function and structure. We speculate that deficits in monoamine systems underlie at least some of the symptoms experienced during withdrawal (for review, see Baumann and Rothman, 1998b).

In particular, we have proposed a dual deficit model of stimulant addiction in which drug-induced DA and 5-HT dysfunction contributes to withdrawal symptoms, drug craving and relapse (Baumann and Rothman, 1998a, b; Rothman

et al., 1998; Baumann et al., 2000). Depicted diagrammatically in Fig. 2, the dual deficit model postulates that decreased synaptic DA during stimulant withdrawal underlies anhedonia and psychomotor retardation, whereas decreased synaptic 5-HT gives rise to depressed mood, obsessive thoughts and lack of impulse control. Consistent with this model, rats receiving repeated injections of abused stimulants exhibit neurobiological changes similar to those observed in human patients with major depression (Markou and Koob, 1991; Baumann et al., 1995a; Baumann and Rothman, 1998a; Lin et al., 1999). If abstinent stimulant addicts exhibit DA and 5-HT deficits, medications capable of correcting abnormalities in DA and 5-HT function might be effective in treating stimulant and alcohol dependence.

In agreement with the dual deficit hypothesis, drugs that release DA (phentermine, amphetamine) or 5-HT (fenfluramine) display properties consistent with the effective treatment of substance use disorders (Rothman et al., 1994, 1998; Yu et al.,

1997; Halladay et al., 1999). For instance, acute or chronic administration of low doses of DA releasers, such as D-amphetamine, decreases cocaine self-administration behaviour in rhesus monkeys (Glowa et al., 1995; Negus and Mello, 2003a, b). The data in Fig. 3 demonstrate that the DA-releasing agent phentermine suppresses responding for cocaine injections without affecting food-reinforced behaviour, and this effect is maintained by daily administration of phentermine (Wojnicki et al., 1999). Such preclinical results provide a rationale for using DA releasers as medications for treating cocaine addiction (Rothman et al., 2002; Grabowski et al., 2004b; Lile, 2006).

Under certain conditions, the 5-HT releaser fenfluramine decreases responding for cocaine in rhesus monkeys as well (Negus et al., 2007). Combined administration of phentermine plus fenfluramine produces a 75% decrease in cocaine self-administration in monkeys (Glowa et al., 1997). The mixture of phentermine and

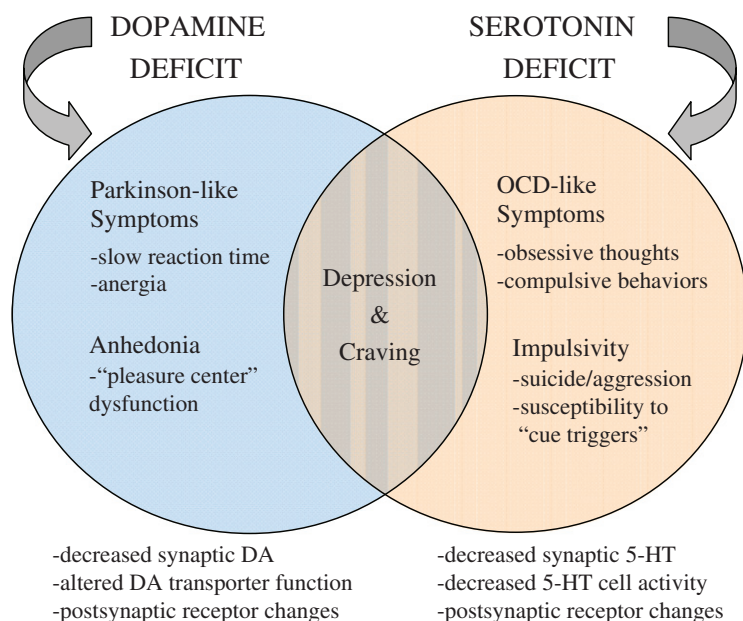


Fig. 2. The dual deficit model of stimulant addiction. According to the model, withdrawal from chronic stimulant use leads to decreased synaptic availability of DA and 5-HT. This dual deficit contributes to withdrawal symptoms, drug craving and relapse. DA dysfunction underlies anhedonia and psychomotor disturbances, whereas 5-HT dysfunction causes depressed mood, obsessive thoughts and lack of impulse control. Protracted withdrawal phenomena are postulated to contribute significantly to relapse. Adapted with permission from Rothman and Baumann (2003).

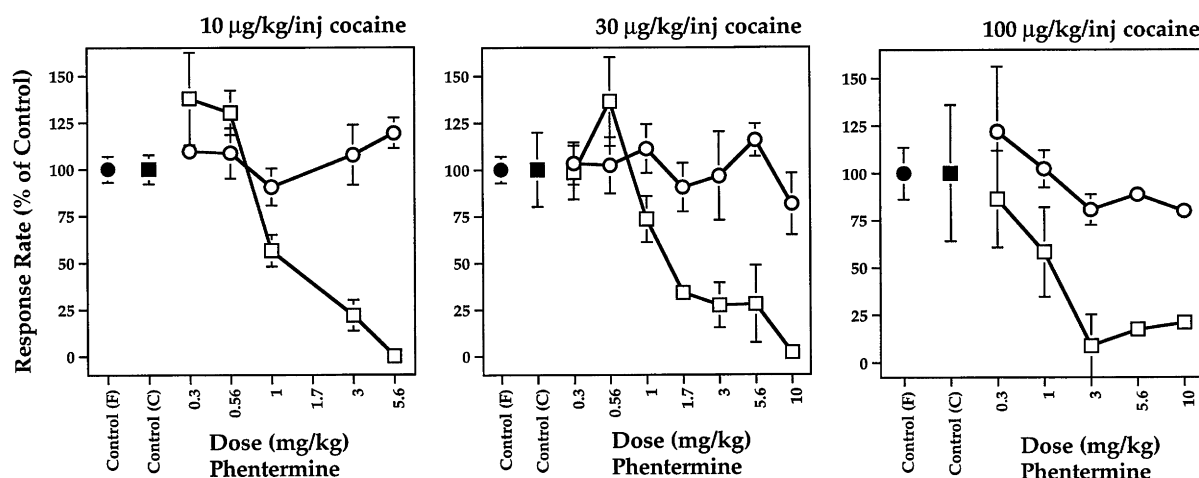


Fig. 3. Acute effects of phentermine on rates of responding maintained under a fixed ratio (FR) 30 schedule of food (○) or cocaine (□) reinforcement. Different unit doses of intravenous cocaine, 10–100 µg/kg/injection, are indicated. Phentermine was administered intravenously. Effects on responding are mean \pm SEM, expressed as percentage of the individual control rates of responding for $N = 3$ –4 monkeys. Control variability (filled symbols) is expressed as the average of individual coefficients of variation. Data taken with permission from Wojnicki et al. (1999).

D-fenfluramine reduces cocaine self-administration by 80% in rats, yet this mixture is not self-administered (Glatz et al., 2002). Importantly, 5-HT-releasing agents suppress cue-elicited cocaine-seeking behaviour in rats (Burmeister et al., 2003) and decrease cocaine craving in cocaine-dependent human patients (Buydens-Branchey et al., 1998). The collective findings suggest that combined treatment with DA and 5-HT releasers may have a greater therapeutic value than treatment with either drug alone, in terms of decreasing stimulant self-administration and reducing cue-induced relapse. Moreover, a growing body of evidence shows that DA/5-HT-releasing agents may provide similar therapeutic benefits for alcohol dependence (Yu et al., 1997; Halladay et al., 1999, 2006).

5-HT release counteracts stimulant effects of DA release

The use of stimulant-like medications to treat stimulant addictions is an approach known as ‘agonist’ therapy. This strategy involves administering medications that are less potent and less addictive than cocaine or methamphetamine, but

that decrease stimulant abuse because of shared neurochemical properties with the abused drugs (Gorelick, 1998). Accordingly, we have described agonist therapy as neurochemical ‘normalization’ therapy — that is, the stimulant medication serves to normalize neurochemical deficits caused by chronic exposure to the abused stimulant (Rothman et al., 2002; Rothman and Baumann, 2003). Neurochemical normalization therapy has generated effective treatments for nicotine dependence (Henningfield, 1995; Rollema et al., 2007) and opioid dependence (Ling et al., 1994; White and Lopatko, 2007). This approach has been explored for the treatment of cocaine dependence as well (Alim et al., 1995; Grabowski et al., 1997; Kampman et al., 2000; Walsh et al., 2000), and a number of placebo-controlled trials have shown promising results (Grabowski et al., 2001, 2004a; Shearer et al., 2003). A significant limitation of this strategy, however, is that candidate medications often exhibit inherent abuse liability due to activation of mesolimbic DA neurons in the brain (for review, see Grabowski et al., 2004b).

One feasible means to decrease the abuse liability of candidate medications is to add 5-HT-releasing properties to these drugs. Several lines of evidence support the hypothesis that elevations in

synaptic 5-HT counteract the stimulant and reinforcing effects mediated by elevations in synaptic DA (Czoty et al., 2002; Daw et al., 2002; Higgins and Fletcher, 2003; Burmeister et al., 2004). Administration of the 5-HT precursor L-tryptophan, which increases 5-HT synthesis and release in the CNS, decreases self-administration of cocaine and amphetamine in rats (Smith et al., 1986; McGregor et al., 1993). Likewise, pretreatment with 5-HT reuptake inhibitors can reduce intravenous cocaine self-administration in rats and squirrel monkeys (Carroll et al., 1990; Howell and Byrd, 1995). Cocaine analogues that have potent affinity at SERT support less self-administration behaviour than analogues with weak affinity for SERT (Roberts et al., 1999; Howell et al., 2007). Consistent with these findings, agents that broadly activate brain 5-HT systems can reduce self-administration of stimulants and other drugs of abuse (Higgins and Fletcher, 2003). The 'anti-stimulant' effects of increasing extracellular 5-HT are readily observed after combined administration of 5-HT and DA releasers, or after administration of single agents that release both neurotransmitters.

As summarized in Table 1, drugs that release [3 H]DA more potently than [3 H]5-HT in vitro (e.g. amphetamine and phentermine) increase endogenous extracellular DA more than extracellular

5-HT in vivo. Such indirect DA agonists are strong locomotor stimulants and support self-administration behaviour. Drugs that release [3 H]5-HT more potently than [3 H]DA in vitro (e.g. fenfluramine and chlorphentermine) increase endogenous extracellular 5-HT more than extracellular DA. Such indirect 5-HT agonists produce minimal motor activity and do not support self-administration behaviour. The anti-stimulant effect of 5-HT releasers is also seen in the conditioned place preference (CPP) assay as shown in Fig. 4, where a low dose of fenfluramine greatly reduces the positive CPP induced by phentermine.

The precise mechanisms responsible for anti-stimulant effects of 5-HT releasers have not been characterized, but increases in extracellular 5-HT would be expected to activate multiple 5-HT receptor subtypes known to modulate DA function (Muller and Huston, 2006; Alex and Pehek, 2007). As mentioned already, stimulant and reinforcing properties of drugs such as cocaine and methamphetamine are mediated via the activation of mesolimbic DA neurons. Cell bodies of mesolimbic DA neurons reside in the midbrain ventral tegmental area (VTA) and send axonal projections to many regions of the forebrain, most notably the nucleus accumbens (Ungerstedt, 1971; Moore and Bloom, 1978). The nucleus accumbens is a critical limbic-motor interface receiving

Table 1. Summary of serotonergic and dopaminergic effects of selected releasing agents

Drug	[3 H]5-HT release EC ₅₀ (nM)	[3 H]DA release EC ₅₀ (nM)	Peak % increase in dialysate 5-HT (dose, mg/kg)	Peak % increase in dialysate DA (dose, mg/kg)	Self-administered	Locomotor activation
Amphetamine ^a	1756	8.0	45 (0.3 mg/kg i.p.)	224 (0.3 mg/kg i.p.)	Yes	Strong
Phentermine ^a	3511	262	32 (1.0 mg/kg i.p.)	156 (1.0 mg/kg i.p.)	Yes	Strong
PAL-353 ^c	1937	24.2	170 (1.0 mg/kg i.v.)	432 (1.0 mg/kg i.v.)	Yes	Strong
Fenfluramine ^a	79.3	> 10,000	215 (1.0 mg/kg i.p.)	20 (1.0 mg/kg i.p.)	No	None
Chlorphentermine ^a	30.9	2650	228 (1.0 mg/kg i.p.)	86 (1.0 mg/kg i.p.)	No	None
Phentermine + fenfluramine ^a	N/A	N/A	222 (1.0 + 1.0 mg/kg i.p.)	144 (1.0 + 1.0 mg/kg i.p.)	No	Weak
PAL-313 ^c	53.4	44.1	544 (1.0 mg/kg i.v.)	130 (1.0 mg/kg i.v.)	Weak	Weak
PAL-287 ^b	3.4	12.6	464 (1.0 mg/kg i.v.)	133 (1.0 mg/kg i.v.)	No	Weak

A summary of data illustrating the tendency for increasing extracellular 5-HT to reduce reinforcing and locomotor effects mediated by increases in extracellular DA. Microdialysis data unpublished. This table originally appeared in Rothman et al. (2007).

^aFrom Baumann et al. (2000) and Rothman et al. (2001).

^bFrom Rothman et al. (2005).

^cFrom Wee et al. (2005).

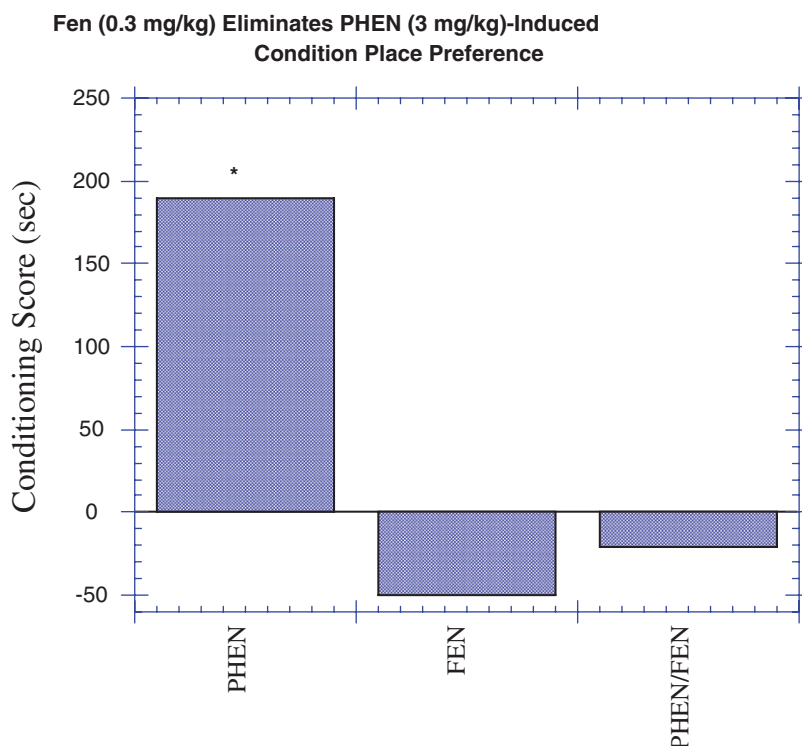


Fig. 4. Effects of phentermine (3 mg/kg) and fenfluramine (0.3 mg/kg), given alone or in combination, on conditioned place preference. Conditioning score represents the mean difference between time (s) spent in the drug vs. vehicle-paired side of the test chamber. All drugs were administered i.p. Each column represents the mean of $N = 9-10$ rats. * = Significant place preference (Wilcoxon test, $P < 0.05$). Data taken with permission from Rea et al. (1998).

afferent inputs from the prefrontal cortex, hippocampus and amygdala, while sending efferent outputs to the ventral pallidum and other regions known to modulate locomotor centres in the brainstem (Mogenson et al., 1980; Pennartz et al., 1994). 5-HT neurons in the midbrain raphe nuclei send axonal projections that densely innervate the mesolimbic system at the level of the VTA and the nucleus accumbens (Steinbusch, 1981; Molliver, 1987). Furthermore, 5-HT neurons innervate various regions providing excitatory afferents to the accumbens (e.g. prefrontal cortex). In this manner, 5-HT nerve terminals are uniquely positioned to influence the activity of mesolimbic DA neurons at multiple levels.

The serotonergic modulation of DA function is inherently complex due to the presence of at least 14 different 5-HT receptor subtypes in the CNS (Barnes and Sharp, 1999; Hoyer et al., 2002).

While most 5-HT receptor subtypes enhance DA transmission (Muller and Huston, 2006; Alex and Pehek, 2007), 5-HT_{2C} receptors provide a strong inhibitory influence on mesolimbic DA neurons (Di Matteo et al., 2001; Bubar and Cunningham, 2006). For instance, systemic administration of the 5-HT_{2C} agonist Ro 60-0175 markedly inhibits DA cell firing in the VTA and decreases extracellular levels of DA in forebrain projection areas (Di Matteo et al., 1999, 2000; Gobert et al., 2000). Pretreatment with Ro 60-0175 reduces locomotor activity and self-administration behaviour produced by cocaine, whereas pretreatment with the 5-HT_{2C} antagonist SB 242084 has the opposite effect (Grottick et al., 2000; Fletcher et al., 2002). In fact, SB 242084 when given alone increases burst firing of DA cells in the VTA, suggesting that 5-HT_{2C} receptors provide tonic inhibition of mesolimbic DA activity. Collectively, these

findings indicate a potential role for 5-HT_{2C} receptors in mediating the anti-stimulant effects of 5-HT releasers (Higgins and Fletcher, 2003).

Recent data show that anti-stimulant effects of 5-HT_{2C} receptor activation involve at least two separate mechanisms — one mechanism in the VTA and another in the prefrontal cortex. Microinjection of Ro 60-0175 into the VTA blocks behavioural effects of cocaine (Fletcher et al., 2004), perhaps reflecting inhibition of DA cell firing and release as noted above (Di Matteo et al., 2001). Microinjection of Ro 60-0175 into the prefrontal cortex also markedly reduces cocaine-induced locomotor activity (Filip and Cunningham, 2003), and this action may involve suppression of excitatory glutamate outputs to the nucleus accumbens (Liu et al., 2007). Neuroanatomical evidence suggests that the effects of 5-HT_{2C} receptor activation in the VTA and cortex are mediated by the stimulation of gamma-aminobutyric acid (GABA) interneurons (Bubar and Cunningham, 2006, 2007; Liu et al., 2007); more research is needed to validate this proposal. Further investigation is warranted to fully elucidate the role of 5-HT_{2C} receptors in mediating anti-stimulant effects of 5-HT releasers. Additionally, the potential of 5-HT_{2C} agonists as medications for substance use disorders deserves to be examined (Higgins and Fletcher, 2003; Bubar and Cunningham, 2006).

Potential adverse effects of 5-HT releasers

The clinical use of 5-HT-releasing agents as medications has been associated with a number of adverse effects (Rothman et al., 1999; Rothman and Baumann, 2002; Zolkowska et al., 2006). Based primarily on experience with D,L-fenfluramine and its more potent isomer D-fenfluramine, three potentially serious side effects need to be considered when 5-HT releasers are developed as treatment agents: valvular heart disease (VHD), idiopathic pulmonary arterial hypertension (IPAH) and neurotoxicity. Fenfluramines were commonly prescribed anorectics until their removal from the market in 1997 due to the occurrence of VHD in some patients (Connolly et al., 1997; Connolly and McGoon, 1999).

Fenfluramine-associated VHD is characterized by thickening of valve leaflets and increased regurgitation of blood, most often detected by echocardiography. While initial findings suggested that fenfluramines induce VHD in a high percentage of patients, more recent evidence shows a much smaller risk. For example, a meta-analysis of available clinical data demonstrates that the incidence of clinically significant valvular regurgitation was 12% in fenfluramine-treated patients vs. 6% in untreated controls (Sachdev et al., 2002).

Because fenfluramines are potent 5-HT releasers (Baumann et al., 2000; Rothman et al., 2003a) and 5-HT has established mitogenic effects (Nemecek et al., 1986; Seuwen et al., 1988), investigators initially speculated that serotonergic mechanisms might contribute to VHD (Connolly et al., 1997; Connolly and McGoon, 1999). To this end, we carried out an investigation to determine whether stereoisomers of fenfluramine, or the *N*-dealkylated metabolite norfenfluramine, might activate mitogenic 5-HT receptors (Rothman et al., 2000a). A number of other test drugs were included in these experiments as positive and negative controls. ‘Positive controls’ were ergot alkaloids known to increase the risk of VHD, such as methysergide, its active metabolite methylergonovine and ergotamine (Bana et al., 1974; Bredberg et al., 1986; Hendriks et al., 1996). ‘Negative controls’ were drugs that interacted with monoamine transporters but did not cause VHD, and these drugs included phentermine, fluoxetine and its metabolite norfluoxetine. We also tested the antidepressant trazodone and its active metabolite *m*-chlorophenylpiperazine (mCPP) as negative controls (Ishida et al., 1995; Otani et al., 1997). mCPP has agonist activity at a wide range of 5-HT receptor subtypes (Hoyer et al., 1994, 2002) and is capable of releasing neuronal 5-HT via a transporter-mediated mechanism similar to that of fenfluramines (Baumann et al., 1993, 2001).

Our working hypothesis was that fenfluramines, norfenfluramines and positive control drugs would share the ability to activate a mitogenic 5-HT receptor subtype expressed in heart valves, while the negative control drugs would not. An initial

receptorome screen led to a detailed evaluation of the binding of these drugs to the 5-HT₂ family of receptors (Rothman et al., 2000a). Table 2 reports binding data, and Table 3 reports the functional effects of these compounds at cloned human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors.

Interestingly, fenfluramines have low affinity for all 5-HT₂ receptor subtypes. By contrast, we found that norfenfluramines displayed high affinity and efficacy at the 5-HT_{2B} receptor subtype ($K_i = 10\text{--}50\text{ nM}$), consistent with the findings of others (Porter et al., 1999; Fitzgerald et al., 2000).

Table 2. K_i values of test drugs at 5-HT₂ receptors

Drug	Human		
	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}
(±)-Fenfluramine	5216 ± 423	4134 ± 1281	3183 ± 637
(+)-Fenfluramine	11,107 ± 2303	5099 ± 1173	6245 ± 874
(-)-Fenfluramine	5463 ± 600	5713 ± 2285	3415 ± 922
(±)-Norfenfluramine	2316 ± 278	52.1 ± 21	557 ± 61
(+)-Norfenfluramine	1516 ± 150	11.2 ± 7.3	324 ± 12
(-)-Norfenfluramine	3841 ± 614	47.8 ± 30.6	814 ± 98
Ergotamine	9.0 ± 1.0	3.0 ± 0.4	12 ± 1.5
Methysergide	15.0 ± 4.0	9.1 ± 4.9	1.8 ± 0.2
Methylergonovine	12.6 ± 1.0	0.49 ± 0.16	12.4 ± 1.0
Fluoxetine	299 ± 53	5030 ± 1960	50 ± 10
Norfluoxetine	638 ± 108	5063 ± 1974	286 ± 60
Trazodone	19.8 ± 2.4	73.6 ± 36	402 ± 44
mCPP	391 ± 47	3.2 ± 1.0	59 ± 11
5-HT	614 ± 74	4.0 ± 1.9	12.2 ± 1.3
Phentermine	> 10,000	> 10,000	> 10,000

Values are mean ± SD for $n = 3$ experiments. Data taken with permission from Rothman et al. (2000a).

Table 3. Functional activity of test drugs at 5-HT₂ receptors

Drug	Human 5-HT _{2A}		Human 5-HT _{2B}		Human 5-HT _{2C}	
	K_{act} (nM ± SD)	V_{max} (percent of 5-HT ± SD)	K_{act} (nM ± SD)	V_{max} (percent of 5-HT ± SD)	K_{act} (nM ± SD)	V_{max} (percent of 5-HT ± SD)
(±)-Fenfluramine	4131 ± 2448	15 ± 4	ND	ND	ND	ND
(+)-Fenfluramine	> 10,000	ND	379 ± 120	38 ± 14	362 ± 109	80 ± 10
(-)-Fenfluramine	5279 ± 998	43 ± 7.2	1248 ± 430	47 ± 5	360 ± 155	84 ± 15
(+)-Norfenfluramine	630 ± 240	88 ± 9	18.4 ± 9	73 ± 6	13 ± 4	100 ± 11
(-)-Norfenfluramine	1565 ± 323	93 ± 9	357 ± 180	71 ± 15	18 ± 9	80 ± 17
Ergotamine	16 ± 4	75 ± 3	9.8 ± 3	56 ± 3	5 ± 3	75 ± 15
Methysergide	3.5 ± 1.7	24 ± 3	150 ± 43	18 ± 4	2.9 ± 1.5	33 ± 3.5
Methylergonovine	1.3 ± 0.4	70 ± 7	0.8 ± 0.5	40 ± 3	2.5 ± 1.2	103 ± 7
Fluoxetine	ND	ND	ND	ND	Antagonist	$K_i = 616 ± 172$
Norfluoxetine	ND	ND	ND	ND	Antagonist	$K_i = 43 ± 17$
Trazodone	Antagonist		Antagonist		Antagonist	
mCPP	65 ± 17	55 ± 11	64 ± 27	43 ± 14	0.64 ± 0.3	79 ± 15
5-HT	66 ± 26	100	2.4 ± 1.5	100	0.6 ± 0.18	100
Phentermine	ND		ND		1394 ± 450	66 ± 10

Values are mean ± SD for $n = 3$ experiments. Data taken with permission from Rothman et al. (2000a).

Methysergide acts as a partial agonist at the 5-HT_{2B} receptor, while the metabolite methylergonovine has even greater affinity and efficacy. Ergotamine is a potent partial agonist at the 5-HT_{2B} receptor. Among the negative control drugs tested, only mCPP exhibits agonist activity at the 5-HT_{2B} site. It is noteworthy that trazodone binds to the 5-HT_{2B} receptor with moderate affinity but functions as an antagonist. Thus, when trazodone is metabolized to mCPP *in vivo* (Ishida et al., 1995; Otani et al., 1997), the 5-HT_{2B} actions of mCPP are probably blocked by antagonist actions of the parent compound.

Our results with the various positive control drugs strongly implicate the 5-HT_{2B} receptor as a major culprit in the development of drug-induced VHD, and accumulating data support this hypothesis (Porter et al., 1999; Fitzgerald et al., 2000; Setola and Roth, 2005; Roth, 2007). 5-HT_{2B} receptors are abundantly expressed on aortic and mitral valves (Fitzgerald et al., 2000), and these receptors are known to stimulate mitogenesis (Lopez-Illasaca, 1998; Hafizi et al., 2000). Further evidence for the role of 5-HT_{2B} receptors in drug-induced VHD is based on the effects of ergot medications such as cabergoline and pergolide. Both of these medications increase the risk of VHD in human patients and are also potent 5-HT_{2B} receptor agonists (for review, see Roth, 2007). Setola et al. (2003) showed that the illicit amphetamine analogue 3,4-methylenedioxymethamphetamine (MDMA) and its *N*-demethylated metabolite, 3,4-methylenedioxymphetamine (MDA), are 5-HT_{2B} receptor agonists. These drugs stimulate prolonged mitogenic responses in human valvular interstitial cells via activation of 5-HT_{2B} receptors (Setola et al., 2003). As predicted by this study, a recent clinical report found that heavy MDMA users display a significantly higher incidence of valvular regurgitation compared to control subjects (Droogmans et al., 2007). More clinical investigations are needed to clearly establish the link between illicit MDMA use and the risk of developing VHD.

Epidemiological evidence indicates that fenfluramines increase the risk of developing IPAH, a debilitating and incurable disease (Abenham et al., 1996; Fishman, 1999). IPAH is characterized

by pulmonary arterial vasoconstriction and hyperplasia that leads to severe hypertension. The pathogenesis of IPAH is complex and difficult to study, especially given the rarity of the disorder. Nonetheless, evidence from our laboratory and others implicates the involvement of 5-HT and SERT proteins in the pathogenesis of IPAH (Rothman et al., 1999; MacLean et al., 2000; Eddahibi et al., 2006). We demonstrated that the SERT substrate activity was a common feature of drugs associated with IPAH, but not all substrates increased the risk of the disease (Rothman et al., 1999). It is well established that blood platelets express SERT proteins identical to those expressed on neurons, and platelet SERT accumulates nearly 99% of circulating 5-HT into platelet storage (Ni and Watts, 2006). One hypothesis — the so-called ‘5-HT hypothesis’ — has been invoked as a potential mechanism underlying fenfluramine-induced IPAH (Fishman, 1999; MacLean et al., 2000). This hypothesis postulates that fenfluramines increase the risk of IPAH by stimulating SERT-mediated release of 5-HT from platelets, thereby elevating plasma levels of 5-HT. Persistent elevations in plasma 5-HT would then cause pulmonary dysfunction. It is noteworthy that an analogous 5-HT hypothesis has been invoked to explain VHD, but as discussed already, drug-induced activation of 5-HT_{2B} receptors seems to be the predominant mechanism involved in this disease.

A key prediction of the 5-HT hypothesis is that fenfluramine increases plasma 5-HT to concentrations sufficient to produce vasoconstriction and mitogenesis, which then leads to serious side effects. Despite the widespread acceptance of the 5-HT hypothesis as an explanation for fenfluramine-associated IPAH, the effects of fenfluramine and related agents on plasma 5-HT have received little attention. Studies conducted in the 1990s do not support the 5-HT hypothesis, since they show that acute fenfluramine does not increase plasma 5-HT in rats and chronic fenfluramine treatment lowers blood 5-HT in humans (Martin and Artigas, 1992; Rothman et al., 2000b). Given the uncertainties regarding validity of the 5-HT hypothesis, we assessed the acute effects of fenfluramine and other amphetamines on

plasma levels of 5-HT in rats (Zolkowska et al., 2006). Specifically, we developed a novel microdialysis method to measure plasma levels of 5-HT in whole blood samples obtained from conscious catheterized rats. Using this method, baseline plasma 5-HT levels in rats were found to be 0.22 nM, or about 1 nM when corrected for dialysis probe recovery, which is similar to plasma 5-HT concentrations measured in human subjects (Herve et al., 1995). Importantly, systemic administration of fenfluramine, MDMA and other amphetamines evokes transient dose-dependent increases in plasma 5-HT ranging from 4 to 20 nM. The ability of drugs to increase plasma 5-HT is directly correlated with their ability to increase SERT-mediated 5-HT release in neurons, suggesting the involvement of platelet SERT proteins. These data show that fenfluramine and other 5-HT releasers are able to acutely increase plasma 5-HT, but the absolute levels of 5-HT are well below the concentrations required to contract pulmonary arteries or stimulate mitogenesis (Cortijo et al., 1997; Eddahibi et al., 1999).

From a medication development standpoint, a more relevant issue is whether chronic administration of 5-HT releasers can persistently increase plasma 5-HT. To address this question, we used microdialysis methods to examine the effects of 2-week minipump infusions of fenfluramine (3 and

10 mg/kg/day) or the 5-HT uptake blocker fluoxetine (3 and 10 mg/kg/day) on plasma levels of 5-HT in rats (Zolkowska et al., 2008). In this study, chronic administration of fenfluramine, but not fluoxetine, caused two- to fourfold increases in baseline dialysate 5-HT levels in blood. Given baseline plasma 5-HT concentrations of about 1 nM, fenfluramine-induced increases in plasma 5-HT are less than 5 nM. The data in Fig. 5 show that chronic exposure to fenfluramine or fluoxetine markedly reduces the ability of acute fenfluramine to evoke increases in plasma 5-HT. Thus, chronic exposure to fenfluramine minimizes the surges in plasma 5-HT caused by acute administration of the drug. Chronic minipump infusions of fenfluramine and fluoxetine in rats give rise to steady-state blood levels of drugs and their bioactive metabolites that are similar to those measured in human patients taking prescribed doses of these medications (Rothman et al., 2000b; Lundmark et al., 2001). Thus, our rat model system is relevant to human patients taking fenfluramine or fluoxetine.

It has been shown that 5-HT provokes contraction of human pulmonary arteries at concentrations ranging from 100 nM to 10 μ M (Cortijo et al., 1997). The threshold concentration of 5-HT required to stimulate mitogenic responses in cultured human pulmonary artery smooth muscle

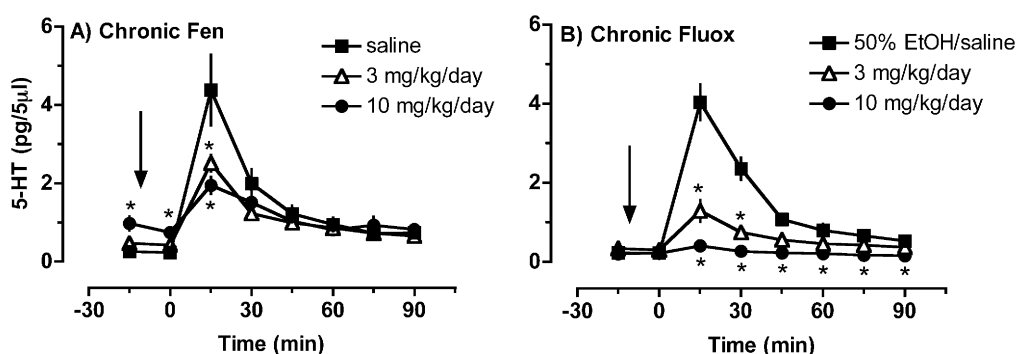


Fig. 5. Effects of acute fenfluramine administration on dialysate 5-HT levels measured in blood from conscious rats previously treated with chronic administration of fenfluramine (Panel A) or fluoxetine (Panel B). For chronic treatments, drugs were dissolved in sterile saline (fenfluramine) or 50% ethanol/saline (fluoxetine) and administered s.c. via osmotic minipumps for 2 weeks. On the day of test, fenfluramine was dissolved in saline and administered i.v. at 0 min. Serial blood samples were withdrawn at 15-min intervals and immediately dialyzed. 5-HT levels are mean \pm SEM for $N = 9$ rats/group. * = $P < 0.05$, compared to saline controls at corresponding time points (Duncan's post hoc test). Data taken with permission from Zolkowska et al. (2008).

cells is about 10 nM (Eddahibi et al., 2001; Marcos et al., 2003), while much higher 5-HT concentrations are needed to stimulate mitogenic responses in rat pulmonary artery smooth muscle cells (Pitt et al., 1994; Eddahibi et al., 1999). Because we found that chronic fenfluramine elevates baseline plasma 5-HT to less than 5 nM in rats, it appears that fenfluramine-induced increases in plasma 5-HT are below the concentrations known to cause pulmonary side effects. Fenfluramine-induced elevations of plasma 5-HT are also much lower than those required to produce VHD in rats exposed to exogenous 5-HT (580–974 nM) (Gustafsson et al., 2005). Viewed collectively, these findings demonstrate that the 5-HT hypothesis cannot explain the mechanism of fenfluramine-associated IPAH or VHD. Of course, it is possible that two- to fourfold increases in plasma 5-HT could be enough to stimulate mitogenic responses in susceptible individuals and increase the risk of developing IPAH. However, this scenario seems unlikely, since treatment with lithium or MAO inhibitors produces two- to fourfold increases in plasma 5-HT without increasing the risk of IPAH (Artigas et al., 1989; Celada et al., 1992).

At present, the mechanisms underlying fenfluramine-associated IPAH remain enigmatic. One significant problem is that most animal models of IPAH require the induction of hypoxia, which is not a major factor in humans. Furthermore, the aetiology of fenfluramine-associated IPAH may differ substantially from that of non-drug-related IPAH. Despite these caveats, recent findings have provided novel hypotheses to explain how fenfluramine might cause IPAH. For example, Launay and colleagues (Launay et al., 2002) have provided evidence that activation of 5-HT_{2B} receptors in the lung is critical to the development of IPAH in a hypoxic mouse model. Since the stereoisomers of norfenfluramine are potent and selective 5-HT_{2B} agonists (Rothman et al., 2000a), a role for 5-HT_{2B} receptors seems feasible. On the other hand, a number of medications that produce VHD and activate 5-HT_{2B} receptors do not increase the risk of IPAH; these medications include methysergide, ergotamine, pergolide and cabergoline.

Additional evidence from our laboratory disputes a role for 5-HT_{2B} sites in fenfluramine-associated IPAH (Rothman and Baumann, 2006). Aminorex is a SERT substrate that caused an epidemic of IPAH in the 1960s (Gurtner, 1985; Fishman, 1999), and case reports implicate the related designer drug 4-methylaminorex as a cause of the disease (Gaine et al., 2000). If 5-HT_{2B} receptors are involved in the pathogenesis of drug-associated IPAH, then one would suspect aminorex to target 5-HT_{2B} sites. While aminorex does interact with cloned human 5-HT_{2B} receptors, the half-maximum effective concentration (EC₅₀) of the drug for 5-HT_{2B} receptor activation (870 nM) is 30-fold higher than its EC₅₀ for NE release (26.4 nM). Moreover, the activity of aminorex at 5-HT_{2B} sites is nearly 50-fold less than that of D-norfenfluramine. It seems plausible that metabolites of aminorex may act more potently at 5-HT_{2B} receptors, and this possibility deserves to be examined. However, the available data argue against an important role for 5-HT_{2B} receptors in the pathogenesis of anorectic-associated IPAH.

Recent studies by Eddahibi et al. (2006) focus on 5-HT produced locally in the lung as a critical player in pathogenesis of IPAH. These investigators reported that endothelial cells in the pulmonary microvasculature synthesize 5-HT, which is then released as a growth factor. It has been proposed that dysregulation of 5-HT production in endothelial cells, along with over-expression of SERT by the pulmonary artery smooth muscle cells, contributes to hyperplasia observed in IPAH. Immunohistochemical studies show that the pulmonary microvascular endothelium does not express SERT (Eddahibi et al., 2006), indicating that fenfluramine cannot release 5-HT from these cells. Based on the findings of Eddahibi et al., administration of the 5-HT precursor L-5-hydroxytryptophan (5-HTP) would be predicted to increase 5-HT synthesis in pulmonary endothelial cells, since this compound bypasses the rate-limiting enzyme tryptophan hydroxylase. 5-HTP is a commonly used dietary supplement with a well-established history of safety (Das et al., 2004), and its use is not known to increase the risk of IPAH. Although speculative, this observation suggests that an increase in 5-HT synthesis in the pulmonary microvascular

endothelial cells may be necessary, but is not sufficient, to increase the risk of IPAH.

Experiments in laboratory animals show that high-dose administration of fenfluramine or *D*-fenfluramine can cause long-term depletion of 5-HT and loss of SERT binding sites in the brain (Zaczek et al., 1990; McCann et al., 1997). The persistent nature of fenfluramine-induced 5-HT deficits in the CNS has been interpreted as evidence for neurotoxicity, although this hypothesis and its clinical relevance are still a matter of debate (Rose et al., 1996; Rothman et al., 2003b). The mechanisms underlying fenfluramine-induced 5-HT depletions are not well understood, but acute 5-HT release has been implicated because 5-HT uptake blockers and synthesis inhibitors can prevent long-term 5-HT depletions (Steranka and Sanders-Bush, 1979; Halladay et al., 2001). An important observation is that not all SERT substrates deplete 5-HT (Nichols et al., 1990; Cozzi et al., 1998; Baumann et al., 2001). As noted previously, mCPP interacts with SERT to release 5-HT from neurons, and mCPP is equipotent with *D*-fenfluramine in this regard (Baumann et al., 1995b, 2001; Eriksson et al., 1999). The data in Fig. 6 demonstrate that repeated high-dose administration of mCPP fails to affect postmortem tissue levels of 5-HT in rat brain whereas

fenfluramine causes profound loss of 5-HT. These data indicate that SERT-mediated 5-HT release is separable from long-term 5-HT depletion.

Elucidating the mechanisms responsible for the adverse effects of 5-HT releasers will have important implications for the future development of SERT substrates as pharmacotherapies. The findings reviewed here provide clues for designing dual DA/5-HT releasers devoid of fenfluramine-like adverse effects. In particular, any lead drug molecule must lack 5-HT_{2B} agonist activity to prevent the risk of VHD. Additionally, candidate drugs should be chemically distinct from the phenylethylamine structure shared by amphetamine-like agents, as non-amphetamine 5-HT releasers have a reduced capacity for causing neurotoxic effects and possibly IPAH.

PAL-287, a non-amphetamine DA/5-HT releaser

Partially based on the above rationale, we sought to identify and characterize a non-amphetamine transporter substrate that would release DA and 5-HT, without affecting release of NE. After an extensive evaluation of over 350 compounds, we found it impossible to dissociate NE- and DA-releasing properties, perhaps due to the

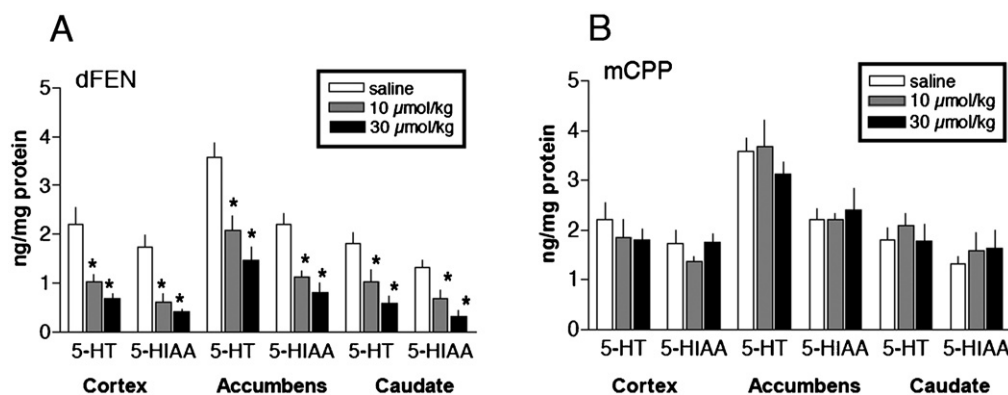


Fig. 6. Effects of high-dose administration of *D*-fenfluramine (*D*-FEN) or mCPP on postmortem tissue levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in rat brain. *D*-FEN or mCPP was administered i.p. at doses of 10 or 30 µmol/kg, every 2 h, for four doses. Rats were killed 2 weeks after the dosing regimen. Postmortem tissue levels of 5-HT and 5-HIAA in the prefrontal cortex, nucleus accumbens and caudate nucleus were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). These doses of *D*-FEN and mCPP produce equivalent increases in extracellular 5-HT. Data are mean ± SEM, expressed as ng/mg protein for *N* = 4–6 rats/group. **P* < 0.05, compared to saline-treated group (Duncan's post hoc test). Data taken with permission from Baumann et al. (2001).

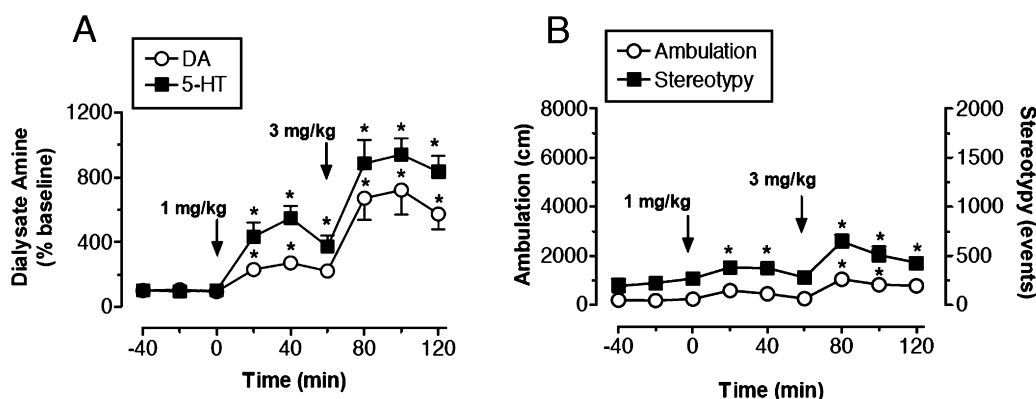


Fig. 7. Effects of PAL-287 on neurochemical and locomotor parameters in rats undergoing in vivo microdialysis in the prefrontal cortex. Rats received i.v. injections of 1 mg/kg PAL-287 at time zero, followed by 3 mg/kg 60 min later. Panel A: Concentrations of DA and 5-HT in dialysate samples are mean \pm SEM for $N = 7$ rats/group, expressed as percentage of the baseline. Baseline levels of DA and 5-HT were 0.43 ± 0.07 and 0.27 ± 0.06 pg/ μ l, respectively. Panel B: Locomotor parameters are mean \pm SEM for $N = 7$ rats/group, expressed as distance traveled in cm (ambulation) and number of repetitive movements (stereotypy). * $P < 0.05$, compared to pre-injection control (Duncan's post hoc test). Data taken with permission from Rothman et al. (2005).

phylogenetic similarities between NET and DAT. The first lead compound from our search was PAL-287 (1-naphthyl-2-aminopropane, see structure in Fig. 1), a novel non-amphetamine monoamine releaser (Rothman et al., 2005). The in vitro potency of PAL-287 at releasing radiolabeled transmitters from DAT, NET and SERT is 12.6 ± 0.4 nM, 11.1 ± 0.9 nM and 3.4 ± 0.2 nM, respectively (see Table 2). Figure 7 shows that administration of PAL-287 to rats increases extracellular 5-HT and DA in a dose-dependent manner, with larger effects on 5-HT compared to DA. Functional studies with cloned human 5-HT_{2B} receptors ($EC_{50} = 40$ nM) and 5-HT_{2A} receptors ($EC_{50} = 466$ nM). The drug is a potent partial agonist at 5-HT_{2C} receptor sites ($EC_{50} = 2.3$ nM, $E_{MAX} = 20\%$), an effect that suggests possible anorectic actions of PAL-287 (Vickers et al., 1999; Nilsson, 2006). 5-HT_{2C} agonist activity may also contribute to the minimal reinforcing properties of PAL-287 despite potent DA-releasing actions of the drug (see Czoty et al., 2002; Higgins and Fletcher, 2003). The weaker potency of PAL-287 at 5-HT_{2A} and 5-HT_{2B} receptors, as compared to its activity at SERT, suggests that the drug may not activate 5-HT_{2A} and 5-HT_{2B} receptors in vivo.

PAL-287 produces minimal locomotor activation despite substantial elevations in extracellular DA (Fig. 7). In particular, the amount of ambulation produced by 3 mg/kg PAL-287 is one-third the amount produced by 1 mg/kg D-amphetamine, even though both drug treatments cause equivalent DA release. These data suggest that 5-HT-releasing properties of PAL-287 limit the stimulant effects of concurrent DA release. Repeated high-dose administration of PAL-287 to rats (18 mg/kg i.p., every 2 h, for three doses) fails to affect brain tissue 5-HT levels when assessed 2 weeks after injections, unlike D-methamphetamine (6.0 mg/kg i.p., every 2 h, for three doses) and MDMA (7.5 mg/kg i.p., every 2 h, for three doses), which cause significant 5-HT depletions. The data in Fig. 8 show that PAL-287 does not support self-administration behaviour, and chronic administration of the drug decreases cocaine self-administration in rhesus monkeys. A dose of 1.0 mg/kg/h PAL-287 significantly reduces both cocaine- and food-maintained responding, but the suppression of cocaine self-administration is somewhat greater than the reduction in food-maintained responding.

Our results with PAL-287 confirm the hypothesis that a non-amphetamine substrate at DAT

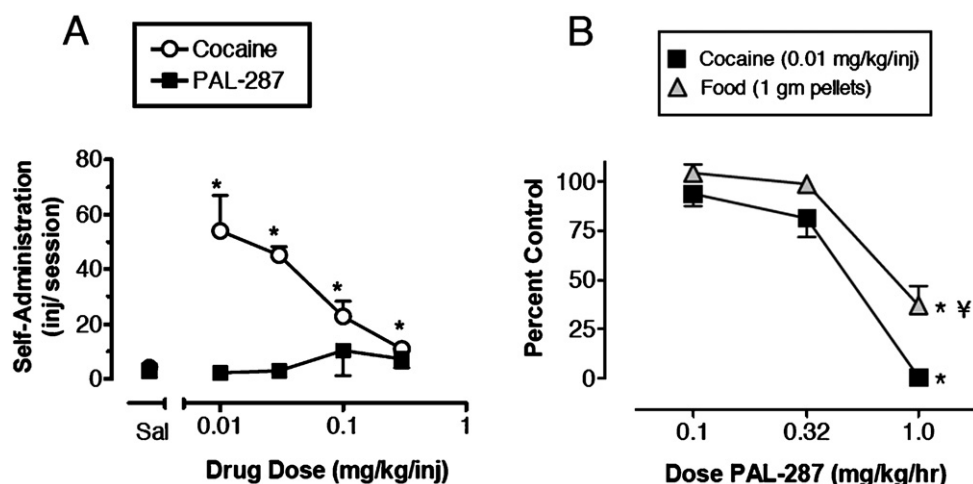


Fig. 8. Effects of PAL-287 in the monkey self-administration assay. Panel A: Self-administration of cocaine and PAL-287 by rhesus monkeys. Drugs were available under a fixed ratio (FR) 25 schedule of reinforcement for 2 h/day. Each point is the mean of two sessions of access to each dose of the drugs. Data are mean \pm SEM for $N = 4$ monkeys. Symbols without bars have variability smaller than the points. $*P < 0.05$, compared to saline-injected control (Newman-Keuls post hoc test). Panel B: Effects of chronic 7-day treatment with PAL-287 on cocaine- and food-maintained responding. Control levels of responding were defined as levels of cocaine- or food-maintained responding observed during 7 days of saline treatment. Each point shows mean \pm SEM for three monkeys, with data collected during the last 3 days of each 7-day treatment. $* = P < 0.05$, compared to control for a given reinforcer (Newman-Keuls post hoc test). $\ddagger = P < 0.05$, compared to cocaine-maintained responding at that dose of PAL-287 (Newman-Keuls post hoc test). Data taken with permission from Rothman et al. (2005).

and SERT will release DA and 5-HT from neurons in vivo, be minimally reinforcing and also suppress ongoing cocaine self-administration. PAL-287 displays a number of desirable qualities for a candidate treatment medication, including minimal locomotor activation, lack of long-term 5-HT neurotoxicity and low abuse potential. Further studies will be necessary to determine the potential of PAL-287 for increasing the risk of VHD and IPAH, especially given the 5-HT_{2B} agonist effects of the drug. The present data with PAL-287 support the use of monoamine releasers as agonist medications for the treatment of stimulant addictions. A dose of 1.0 mg/kg/h PAL-287 virtually eliminated cocaine self-administration in rhesus monkeys by the end of the 7-day treatment, although this effect was not entirely selective for cocaine vs. food. We also note that the role of NE in the actions of PAL-287 is an important issue awaiting additional study (Rothman et al., 2001).

Conclusions

Our findings with PAL-287 in monkeys are similar to the suppression of cocaine self-administration produced by D-amphetamine, although D-amphetamine displays greater selectivity in reducing cocaine self-administration as opposed to food-maintained responding (Negus and Mello, 2003a). Grabowski et al. (2001, 2004b) showed that a slow-release formulation of D-amphetamine is effective in maintaining cocaine addicts in treatment and reducing illicit cocaine use. We predict that agents such as PAL-287, which have mixed DA/5-HT-releasing activity, will possess the therapeutic effects of amphetamine-type monoamine releasers, while minimizing the adverse effects associated with the phenethylamine structure. Based on observations that dual DA/5-HT releasers suppress alcohol ingestion (Yu et al., 1997; Halladay et al., 1999, 2006), it seems that PAL-287 or similar agents should be tested as potential treatments for

alcohol addiction. Additionally, combined treatment with DA and 5-HT releasers blocks alcohol withdrawal seizures (Yu et al., 1997). Although further work remains to refine PAL-287, in particular to reduce its potency at 5-HT_{2B} receptors, we believe that PAL-287 represents the prototype for a new generation of drugs that enhance monoamine release by acting as substrates at multiple transporters.

While compounds such as PAL-287 move slowly from the preclinical arena towards clinical development, it is possible to test the concept of administering dual DA/5-HT releasers in humans by implementing clinically available compounds. For example, the utility of DA/5-HT releasers as treatments for addictive disorders can be tested by administration of the DA releaser D-amphetamine along with the 5-HT precursor 5-HTP. It is noteworthy that 5-HTP must be co-administered with the peripheral decarboxylase inhibitor carbidopa to selectively increase extracellular 5-HT in the CNS (see Halladay et al., 2006). Moreover, the utility of DA/5-HT releasers could also be tested using phentermine and 5-HTP/carbidopa, a drug combination with predicted efficacy as an appetite suppressant (Rothman and Baumann, 2008). In summary, we suggest that drugs with a mode of action similar to that of PAL-287 will provide neurochemical normalization therapy for stimulant addictions and might also be useful for treating depression, obsessive compulsive disorder, attention deficit hyperactivity disorder and obesity.

Abbreviations

CNS	central nervous system
CPP	conditioned place preference
DA	dopamine
DAT	dopamine transporter
EC ₅₀	half-maximal effective concentration
E _{MAX}	maximal efficacy
GABA	gamma-aminobutyric acid
5-HT	5-hydroxytryptamine or serotonin
5-HTP	L-5-hydroxytryptophan

IPAH	idiopathic pulmonary arterial hypertension
mCPP	<i>m</i> -chlorophenylpiperazine
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
NE	norepinephrine
NET	norepinephrine transporter
PAL-287	1-naphthyl-2-aminopropane
PAL-313	<i>p</i> -methylanphetamine
PAL-353	<i>m</i> -fluoroamphetamine
Ro 60-0175	(S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine
SB 242084	6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbonyl]-indoline
SERT	serotonin transporter
VHD	valvular heart disease
VMAT2	vesicular monoamine transporter type 2
MTA	ventral tegmental area

Acknowledgements

This research was supported in part by the Intramural Research Program of the NIH, NIDA and NIDA R01 DA12970 to Bruce Blough.

References

- Abenhaim, L., Moride, Y., Brenot, F., Rich, S., Benichou, J., Kurz, X., Higenbottam, T., Oakley, C., Wouters, E., Aubier, M., Simonneau, G. and Begaud, B. (1996) Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N. Engl. J. Med.*, 335(9): 609–616.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113(2): 296–320.
- Alexander, M., Rothman, R.B., Baumann, M.H., Endres, C.J., Brasic, J.R. and Wong, D.F. (2005) Noradrenergic and dopaminergic effects of (+)-amphetamine-like stimulants in the baboon *Papio anubis*. *Synapse*, 56(2): 94–99.
- Alim, T.N., Rosse, R.B., Vocci, F.J., Jr., Lindquist, T. and Deutsch, S.I. (1995) Diethylpropion pharmacotherapeutic adjuvant therapy for inpatient treatment of cocaine dependence: a test of the cocaine-agonist hypothesis. *Clin. Neuropharmacol.*, 18(2): 183–195.
- Amara, S.G. and Kuhar, M.J. (1993) Neurotransmitter transporters: recent progress. *Annu. Rev. Neurosci.*, 16: 73–93.

- Amara, S.G. and Sonders, M.S. (1998) Neurotransmitter transporters as molecular targets for addictive drugs. *Drug Alcohol Depend.*, 51(1–2): 87–96.
- Anonymous. (1995) Increasing morbidity and mortality associated with abuse of methamphetamine — United States, 1991–1994. *Morb. Mortal. Wkly. Rep.*, 44(47): 882–886.
- Arnsten, A.F. (2006) Stimulants: therapeutic actions in ADHD. *Neuropsychopharmacology*, 31(11): 2376–2383.
- Artigas, F., Sarrias, M.J., Martinez, E., Gelpi, E., Alvarez, E. and Udina, C. (1989) Increased plasma free serotonin but unchanged platelet serotonin in bipolar patients treated chronically with lithium. *Psychopharmacology (Berl.)*, 99(3): 328–332.
- Bana, D.S., Macneal, P.S., Lecompte, P.M., Shah, Y. and Graham, J.R. (1974) Cardiac murmurs and endocardial fibrosis associated with methysergide therapy. *Am. Heart J.*, 88(5): 640–655.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Baumann, M.H., Ayestas, M.A., Dersch, C.M., Brockington, A., Rice, K.C. and Rothman, R.B. (2000) Effects of phentermine and fenfluramine on extracellular dopamine and serotonin in rat nucleus accumbens: therapeutic implications. *Synapse*, 36(2): 102–113.
- Baumann, M.H., Ayestas, M.A., Dersch, C.M. and Rothman, R.B. (2001) 1-(m-Chlorophenyl)piperazine (mCPP) dissociates in vivo serotonin release from long-term serotonin depletion in rat brain. *Neuropsychopharmacology*, 24(5): 492–501.
- Baumann, M.H., Becketts, K.M. and Rothman, R.B. (1995a) Evidence for alterations in presynaptic serotonergic function during withdrawal from chronic cocaine in rats. *Eur. J. Pharmacol.*, 282(1–3): 87–93.
- Baumann, M.H., Mash, D.C. and Staley, J.K. (1995b) The serotonin agonist m-chlorophenylpiperazine (mCPP) binds to serotonin transporter sites in human brain. *Neuroreport*, 6(16): 2150–2152.
- Baumann, M.H. and Rothman, R.B. (1998a) Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. *Biol. Psychiatry*, 44(7): 578–591.
- Baumann, M.H. and Rothman, R.B. (1998b) Serotonergic dysfunction during cocaine withdrawal: implications for cocaine-induced depression. In: Karch S.B. (Ed.), *Drug Abuse Handbook*. CRC Press, Boca Raton, pp. 463–484.
- Baumann, M.H., Rutter, J.J. and Auerbach, S.B. (1993) Intravenous administration of the serotonin agonist m-chlorophenylpiperazine (mCPP) increases extracellular serotonin in the diencephalon of awake rats. *Neuropharmacology*, 32(12): 1381–1386.
- Blakely, R.D., Defelice, L.J. and Galli, A. (2005) Biogenic amine neurotransmitter transporters: just when you thought you knew them. *Physiology (Bethesda)*, 20(4): 225–231.
- Bredberg, U., Eyjolfsson, G.S., Paalzow, L., Tfelt-Hansen, P. and Tfelt-Hansen, V. (1986) Pharmacokinetics of methysergide and its metabolite methylethergometrine in man. *Eur. J. Clin. Pharmacol.*, 30(1): 75–77.
- Bubar, M.J. and Cunningham, K.A. (2006) Serotonin 5-HT_{2A} and 5-HT_{2C} receptors as potential targets for modulation of psychostimulant use and dependence. *Curr. Top. Med. Chem.*, 6(18): 1971–1985.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience*, 146(1): 286–297.
- Burmeister, J.J., Lungren, E.M., Kirschner, K.F. and Neisewander, J.L. (2004) Differential roles of 5-HT receptor subtypes in cue and cocaine reinstatement of cocaine-seeking behavior in rats. *Neuropsychopharmacology*, 29(4): 660–668.
- Burmeister, J.J., Lungren, E.M. and Neisewander, J.L. (2003) Effects of fluoxetine and D-fenfluramine on cocaine-seeking behavior in rats. *Psychopharmacology*, 168(1–2): 146–154.
- Buydens-Branchey, L., Branchey, M., Hudson, J., Rothman, M., Ferguson, P. and Mckernin, C. (1998) Effect of fenfluramine challenge on cocaine craving in addicted male users. *Am. J. Addict.*, 7(2): 142–155.
- Carroll, M.E., Lac, S.T., Asencio, M. and Kragh, R. (1990) Fluoxetine reduces intravenous cocaine self-administration in rats. *Pharmacol. Biochem. Behav.*, 35(1): 237–244.
- Celada, P., Dolera, M., Alvarez, E. and Artigas, F. (1992) Effects of acute and chronic treatment with fluvoxamine on extracellular and platelet serotonin in the blood of major depressive patients. Relationship to clinical improvement. *J. Affect. Disord.*, 25(4): 243–249.
- Connolly, H.M., Cray, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S. and Schaff, H.V. (1997) Valvular heart disease associated with fenfluramine-phentermine. *N. Engl. J. Med.*, 337(9): 581–588.
- Connolly, H.M. and McGoon, M.D. (1999) Obesity drugs and the heart. *Curr. Probl. Cardiol.*, 24(12): 745–792.
- Cortijo, J., Marti-Cabrera, M., Bernabeu, E., Domenech, T., Bou, J., Fernandez, A.G., Beleta, J., Palacios, J.M. and Morcillo, E.J. (1997) Characterization of 5-HT receptors on human pulmonary artery and vein: functional and binding studies. *Br. J. Pharmacol.*, 122(7): 1455–1463.
- Cozzi, N.V., Frescas, S., Marona-Lewicka, D., Huang, X. and Nichols, D.E. (1998) Indan analogs of fenfluramine and norfenfluramine have reduced neurotoxic potential. *Pharmacol. Biochem. Behav.*, 59(3): 709–715.
- Czoty, P.W., Ginsburg, B.C. and Howell, L.L. (2002) Serotonergic attenuation of the reinforcing and neurochemical effects of cocaine in squirrel monkeys. *J. Pharmacol. Exp. Ther.*, 300(3): 831–837.
- Dackis, C.A. and Gold, M.S. (1985) New concepts in cocaine addiction: the dopamine depletion hypothesis. *Neurosci. Biobehav. Rev.*, 9(3): 469–477.
- Das, G. (1993) Cocaine abuse in North America: a milestone in history. *J. Clin. Pharmacol.*, 33(4): 296–310.
- Das, Y.T., Bagchi, M., Bagchi, D. and Preuss, H.G. (2004) Safety of 5-hydroxy-L-tryptophan. *Toxicol. Lett.*, 150(1): 111–122.
- Daw, N.D., Kakade, S. and Dayan, P. (2002) Opponent interactions between serotonin and dopamine. *Neural Netw.*, 15(4–6): 603–616.

- Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., Acquas, E., Carboni, E., Valentini, V. and Lecca, D. (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology*, 47(Suppl. 1): 227–241.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22(5): 229–232.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38(8): 1195–1205.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin_{2C} receptors. *Brain Res.*, 865(1): 85–90.
- Droogmans, S., Cosyns, B., D'haenen, H., Creten, E., Weytjens, C., Franken, P.R., Scott, B., Schoors, D., Kemdem, A., Close, L., Vandenbossche, J.-L., Bechet, S. and Van Camp, G. (2007) Possible association between 3,4-methylenedioxymethamphetamine abuse and valvular heart disease. *Am. J. Cardiol.*, 100(9): 1442–1445.
- Eddahibi, S., Fabre, V., Boni, C., Martres, M.P., Raffestin, B., Hamon, M. and Adnot, S. (1999) Induction of serotonin transporter by hypoxia in pulmonary vascular smooth muscle cells. Relationship with the mitogenic action of serotonin. *Circ. Res.*, 84(3): 329–336.
- Eddahibi, S., Guignabert, C., Barlier-Mur, A.M., Dewachter, L., Fadel, E., Darteville, P., Humbert, M., Simonneau, G., Hanoun, N., Saurini, F., Hamon, M. and Adnot, S. (2006) Cross talk between endothelial and smooth muscle cells in pulmonary hypertension: critical role for serotonin-induced smooth muscle hyperplasia. *Circulation*, 113(15): 1857–1864.
- Eddahibi, S., Humbert, M., Fadel, E., Raffestin, B., Darmon, M., Capron, F., Simonneau, G., Darteville, P., Hamon, M. and Adnot, S. (2001) Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J. Clin. Invest.*, 108(8): 1141–1150.
- Eriksson, E., Engberg, G., Bing, O. and Nissbrandt, H. (1999) Effects of mCPP on the extracellular concentrations of serotonin and dopamine in rat brain. *Neuropsychopharmacology*, 20(3): 287–296.
- Filip, M. and Cunningham, K.A. (2003) Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin_{2C} (5-HT_{2C}) receptors in rat prefrontal cortex. *J. Pharmacol. Exp. Ther.*, 306(2): 734–743.
- Fishman, A.P. (1999) Aminorex to fen/phen: an epidemic foretold. *Circulation*, 99(1): 156–161.
- Fitzgerald, L.W., Burn, T.C., Brown, B.S., Patterson, J.P., Corjay, M.H., Valentine, P.A., Sun, J.H., Link, J.R., Abbaszade, I., Hollis, J.M., Largent, B.L., Hartig, P.R., Hollis, G.F., Meunier, P.C., Robichaud, A.J. and Robertson, D.W. (2000) Possible role of valvular serotonin 5-HT_{2B} receptors in the cardiopathy associated with fenfluramine. *Mol. Pharmacol.*, 57(1): 75–81.
- Fleckenstein, A.E., Volz, T.J., Riddle, E.L., Gibb, J.W. and Hanson, G.R. (2007) New insights into the mechanism of action of amphetamines. *Annu. Rev. Pharmacol. Toxicol.*, 47: 681–698.
- Fletcher, P.J., Chintoh, A.F., Sinyard, J. and Higgins, G.A. (2004) Injection of the 5-HT_{2C} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. *Neuropsychopharmacology*, 29(2): 308–318.
- Fletcher, P.J., Grottick, A.J. and Higgins, G.A. (2002) Differential effects of the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27(4): 576–586.
- Gainé, S.P., Rubin, L.J., Kmetzo, J.J., Palevsky, H.I. and Traill, T.A. (2000) Recreational use of aminorex and pulmonary hypertension. *Chest*, 118(5): 1496–1497.
- Garlow, S.J., Purselle, D. and D'orio, B. (2003) Cocaine use disorders and suicidal ideation. *Drug Alcohol Depend.*, 70(1): 101–104.
- Gawin, F.H. and Kleber, H.D. (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. *Arch. Gen. Psychiatry*, 43(2): 107–113.
- Ghitza, U.E., Rothman, R.B., Gorelick, D.A., Henningfield, J.E. and Baumann, M.H. (2007) Serotonergic responsiveness in human cocaine users. *Drug Alcohol Depend.*, 86(2–3): 207–213.
- Glatz, A.C., Ehrlich, M., Bae, R.S., Clarke, M.J., Quinlan, P.A., Brown, E.C., Rada, P. and Hoebel, B.G. (2002) Inhibition of cocaine self-administration by fluoxetine or D-fenfluramine combined with phentermine. *Pharmacol. Biochem. Behav.*, 71(1–2): 197–204.
- Glowa, J.R., Rice, K.C., Matecka, D. and Rothman, R.B. (1997) Phentermine/fenfluramine decreases cocaine self-administration in rhesus monkeys. *Neuroreport*, 8(6): 1347–1351.
- Glowa, J.R., Wojnicki, F.H.E., Matecka, D., Rice, K.C. and Rothman, R.B. (1995) Effects of dopamine reuptake inhibitors on food- and cocaine-maintained responding: II: comparisons with other drugs and repeated administrations. *Exp. Clin. Psychopharmacol.*, 3(4): 232–239.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36(3): 205–221.
- Gonzalez Castro, F., Barrington, E.H., Walton, M.A. and Rawson, R.A. (2000) Cocaine and methamphetamine: differential addiction rates. *Psychol. Addict. Behav.*, 14(4): 390–396.
- Gorelick, D.A. (1998) The rate hypothesis and agonist substitution approaches to cocaine abuse treatment. *Adv. Pharmacol.*, 42: 995–997.
- Grabowski, J., Roache, J.D., Schmitz, J.M., Rhoades, H., Creson, D. and Korszun, A. (1997) Replacement medication

- for cocaine dependence: methylphenidate. *J. Clin. Psychopharmacol.*, 17(6): 485–488.
- Grabowski, J., Rhoades, H., Schmitz, J., Stotts, A., Daruszka, L.A., Creson, D. and Moeller, F.G. (2001) Dextroamphetamine for cocaine-dependence treatment: a double-blind randomized clinical trial. *J. Clin. Psychopharmacol.*, 21(5): 522–526.
- Grabowski, J., Rhoades, H., Stotts, A., Cowan, K., Kopecky, C., Dougherty, A., Moeller, F.G., Hassan, S. and Schmitz, J. (2004a) Agonist-like or antagonist-like treatment for cocaine dependence with methadone for heroin dependence: two double-blind randomized clinical trials. *Neuropsychopharmacology*, 29(5): 969–981.
- Grabowski, J., Shearer, J., Merrill, J. and Negus, S.S. (2004b) Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict. Behav.*, 29(7): 1439–1464.
- Greenhill, L.L. (2006) The science of stimulant abuse. *Pediatr. Ann.*, 35(8): 552–556.
- Grothick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT(2C) receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295(3): 1183–1191.
- Gurtner, H.P. (1985) Aminorex and pulmonary hypertension. *Cor Vasa*, 27(2–3): 160–171.
- Gustafsson, B.I., Tommeras, K., Nordrum, I., Loennechen, J.P., Brunsvik, A., Solligard, E., Fossmark, R., Bakke, I., Syversen, U. and Waldum, H. (2005) Long-term serotonin administration induces heart valve disease in rats. *Circulation*, 111(12): 1517–1522.
- Hafizi, S., Taylor, P.M., Chester, A.H., Allen, S.P. and Yacoub, M.H. (2000) Mitogenic and secretory responses of human valve interstitial cells to vasoactive agents. *J. Heart Valve Dis.*, 9(3): 454–458.
- Halladay, A.K., Kirschner, E., Hesse, K., Fisher, H. and Wagner, G.C. (2001) Role of monoamine oxidase inhibition and monoamine depletion in fenfluramine-induced neurotoxicity and serotonin release. *Pharmacol. Toxicol.*, 89(5): 237–248.
- Halladay, A.K., Wagner, G.C., Hsu, T., Sekowski, A. and Fisher, H. (1999) Differential effects of monoaminergic agonists on alcohol intake in rats fed a tryptophan-enhanced diet. *Alcohol*, 18(1): 55–64.
- Halladay, A.K., Wagner, G.C., Sekowski, A., Rothman, R.B., Baumann, M.H. and Fisher, H. (2006) Alterations in alcohol consumption, withdrawal seizures, and monoamine transmission in rats treated with phentermine and 5-hydroxy-L-tryptophan. *Synapse*, 59(5): 277–289.
- Haney, M., Ward, A.S., Gerra, G. and Foltin, R.W. (2001) Neuroendocrine effects of D-fenfluramine and bromocriptine following repeated smoked cocaine in humans. *Drug Alcohol Depend.*, 64(1): 63–73.
- Hendriks, M., Van Dorpe, J., Flameng, W. and Daenen, W. (1996) Aortic and mitral valve disease induced by ergotamine therapy for migraine: a case report and review of the literature. *J. Heart Valve Dis.*, 5(2): 235–237.
- Henningfield, J.E. (1995) Nicotine medications for smoking cessation. *N. Engl. J. Med.*, 333(18): 1196–1203.
- Herve, P., Launay, J.-M., Scrobohaci, M.-L., Brenot, F., Simonneau, G., Petitpretz, P., Poubeau, P., Cerrina, J., Duroux, P. and Drouet, L. (1995) Increased plasma serotonin in primary pulmonary hypertension. *Am. J. Med.*, 99(3): 249–254.
- Higgins, G.A. and Fletcher, P.J. (2003) Serotonin and drug reward: focus on 5-HT_{2C} receptors. *Eur. J. Pharmacol.*, 480(1–3): 151–162.
- Howell, L.L. and Byrd, L.D. (1995) Serotonergic modulation of the behavioral effects of cocaine in the squirrel monkey. *J. Pharmacol. Exp. Ther.*, 275(3): 1551–1559.
- Howell, L.L., Carroll, F.I., Votaw, J.R., Goodman, M.M. and Kimmel, H.L. (2007) Effects of combined dopamine and serotonin transporter inhibitors on cocaine self-administration in rhesus monkeys. *J. Pharmacol. Exp. Ther.*, 320(2): 757–765.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, 46(2): 157–203.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Hyman, S.E. (2005) Addiction: a disease of learning and memory. *Am. J. Psychiatry*, 162(8): 1414–1422.
- Ishida, M., Otani, K., Kaneko, S., Ohkubo, T., Osanai, T., Yasui, N., Mihara, K., Higuchi, H. and Sugawara, K. (1995) Effects of various factors on steady state plasma concentrations of trazodone and its active metabolite m-chlorophenylpiperazine. *Int. Clin. Psychopharmacol.*, 10(3): 143–146.
- Iversen, L. (2006) Neurotransmitter transporters and their impact on the development of psychopharmacology. *Br. J. Pharmacol.*, 147(S1): S82–S88.
- Kalivas, P.W. and O'Brien, C. (2008) Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology*, 33(1): 166–180.
- Kampan, K.M., Rukstalis, M., Pettinati, H., Muller, E., Acosta, T., Gariti, P., Ehrman, R. and O'Brien, C.P. (2000) The combination of phentermine and fenfluramine reduced cocaine withdrawal symptoms in an open trial. *J. Subst. Abuse Treat.*, 19(1): 77–79.
- Koob, G.F. (2003) Alcoholism: allostasis and beyond. *Alcohol. Clin. Exp. Res.*, 27(2): 232–243.
- Koob, G.F., Roberts, A.J., Schulteis, G., Parsons, L.H., Heyser, C.J., Hyttia, P., Merlo-Pich, E. and Weiss, F. (1998) Neurocircuitry targets in ethanol reward and dependence. *Alcohol. Clin. Exp. Res.*, 22(1): 3–9.
- Launay, J.M., Herve, P., Peoc'h, K., Tournais, C., Callebort, J., Nebigil, C.G., Etienne, N., Drouet, L., Humbert, M., Simonneau, G. and Maroteaux, L. (2002) Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat. Med.*, 8(10): 1129–1135.
- Lesch, K.P. (2005) Alcohol dependence and gene x environment interaction in emotion regulation: is serotonin the link? *Eur. J. Pharmacol.*, 526(1–3): 113–124.
- Levy, A.D., Li, Q. and Van De Kar, L.D. (1994) Repeated cocaine exposure inhibits the adrenocorticotrophic hormone

- response to the serotonin releaser D-fenfluramine and the 5-HT_{1A} agonist, 8-OH-DPAT. *Neuropharmacology*, 33(3–4): 335–342.
- Lile, J.A. (2006) Pharmacological determinants of the reinforcing effects of psychostimulants: relation to agonist substitution treatment. *Exp. Clin. Psychopharmacol.*, 14(1): 20–33.
- Lin, D., Koob, G.F. and Markou, A. (1999) Differential effects of withdrawal from chronic amphetamine or fluoxetine administration on brain stimulation reward in the rat — interactions between the two drugs. *Psychopharmacology*, 145(3): 283–294.
- Ling, W., Rawson, R.A. and Compton, M.A. (1994) Substitution pharmacotherapies for opioid addiction: from methadone to LAAM and buprenorphine. *J. Psychoactive Drugs*, 26(2): 119–128.
- Liu, S., Bubar, M.J., Lanfranco, M.F., Hillman, G.R. and Cunningham, K.A. (2007) Serotonin_{2C} receptor localization in GABA neurons of the rat medial prefrontal cortex: implications for understanding the neurobiology of addiction. *Neuroscience*, 146(4): 1677–1688.
- Lopez-Illasaca, M. (1998) Signaling from G-protein-coupled receptors to mitogen-activated protein (MAP)-kinase cascades. *Biochem. Pharmacol.*, 56(3): 269–277.
- Lundmark, J., Reis, M. and Bengtsson, F. (2001) Serum concentrations of fluoxetine in the clinical treatment setting. *Ther. Drug Monit.*, 23(2): 139–147.
- Maclean, M.R., Herve, P., Eddahibi, S. and Adnot, S. (2000) 5-hydroxytryptamine and the pulmonary circulation: receptors, transporters and relevance to pulmonary arterial hypertension. *Br. J. Pharmacol.*, 131(2): 161–168.
- Mann, J.J. (2003) Neurobiology of suicidal behaviour. *Nat. Rev. Neurosci.*, 4(10): 819–828.
- Marcos, E., Adnot, S., Pham, M.H., Nosjean, A., Raffestin, B., Hamon, M. and Eddahibi, S. (2003) Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. *Am. J. Respir. Crit. Care Med.*, 168(4): 487–493.
- Markou, A. and Koob, G.F. (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology*, 4(1): 17–26.
- Martin, F. and Artigas, F. (1992) Simultaneous effects of p-chloroamphetamine, D-fenfluramine, and reserpine on free and stored 5-hydroxytryptamine in brain and blood. *J. Neurochem.*, 59(3): 1138–1144.
- Martinez, D., Narendran, R., Foltin, R.W., Slifstein, M., Hwang, D.R., Broft, A., Huang, Y., Cooper, T.B., Fischman, M.W., Kleber, H.D. and Laruelle, M. (2007) Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. *Am. J. Psychiatry*, 164(4): 622–629.
- Masson, J., Sagne, C., Hamon, M. and El Mestikawy, S. (1999) Neurotransmitter transporters in the central nervous system. *Pharmacol. Rev.*, 51(3): 439–464.
- McCann, U.D., Seiden, L.S., Rubin, L.J. and Ricaurte, G.A. (1997) Brain serotonin neurotoxicity and primary pulmonary hypertension from fenfluramine and dexfenfluramine. A systematic review of the evidence. *J. Am. Med. Assoc.*, 278(8): 666–672.
- McGregor, A., Lacosta, S. and Roberts, D.C. (1993) L-tryptophan decreases the breaking point under a progressive ratio schedule of intravenous cocaine reinforcement in the rat. *Pharmacol. Biochem. Behav.*, 44(3): 651–655.
- Mogenson, G.J., Jones, D.L. and Yim, C.Y. (1980) From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol.*, 14(2–3): 69–97.
- Molliver, M.E. (1987) Serotonergic neuronal systems: what their anatomic organization tells us about function. *J. Clin. Psychopharmacol.*, 7(6 Suppl): 3S–23S.
- Moore, R.Y. and Bloom, F.E. (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. *Annu. Rev. Neurosci.*, 1: 129–169.
- Muller, C.P. and Huston, J.P. (2006) Determining the region-specific contributions of 5-HT receptors to the psychostimulant effects of cocaine. *Trends Pharmacol. Sci.*, 27(2): 105–112.
- Musto, D.F. (1992) Cocaine's history, especially the American experience. *Ciba Found. Symp.*, 166: 7–14.
- Negus, S.S. and Mello, N.K. (2003a) Effects of chronic D-amphetamine treatment on cocaine- and food-maintained responding under a progressive-ratio schedule in rhesus monkeys. *Psychopharmacology*, 167(3): 324–332.
- Negus, S.S. and Mello, N.K. (2003b) Effects of chronic D-amphetamine treatment on cocaine- and food-maintained responding under a second-order schedule in rhesus monkeys. *Drug Alcohol Depend.*, 70(1): 39–52.
- Negus, S.S., Mello, N.K., Blough, B.E., Baumann, M.H. and Rothman, R.B. (2007) Monoamine releasers with varying selectivity for dopamine/norepinephrine versus serotonin release as candidate “agonist” medications for cocaine dependence: studies in assays of cocaine discrimination and cocaine self-administration in rhesus monkeys. *J. Pharmacol. Exp. Ther.*, 320(2): 627–636.
- Nemecek, G.M., Coughlin, S.R., Handley, D.A. and Moskowitz, M.A. (1986) Stimulation of aortic smooth muscle cell mitogenesis by serotonin. *Proc. Natl. Acad. Sci. U.S.A.*, 83(3): 674–678.
- Ni, W. and Watts, S.W. (2006) 5-hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). *Clin. Exp. Pharmacol. Physiol.*, 33(7): 575–583.
- Nichols, D.E., Brewster, W.K., Johnson, M.P., Oberlander, R. and Riggs, R.M. (1990) Nonneurotoxic tetralin and indan analogues of 3,4-(methylenedioxy)amphetamine (MDA). *J. Med. Chem.*, 33(2): 703–710.
- Nilsson, B.M. (2006) 5-Hydroxytryptamine 2C (5-HT_{2C}) receptor agonists as potential antiobesity agents. *J. Med. Chem.*, 49(14): 4023–4034.
- Otani, K., Mihara, K., Yasui, N., Ishida, M., Kondo, T., Tokinaga, N., Ohkubo, T., Osanai, T., Sugawara, K. and Kaneko, S. (1997) Plasma concentrations of trazodone and m-chlorophenylpiperazine at steady state can be predicted from those after an initial dose of trazodone. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 21(1): 239–244.
- Parsons, L.H., Koob, G.F. and Weiss, F. (1995) Serotonin dysfunction in the nucleus accumbens of rats during

- withdrawal after unlimited access to intravenous cocaine. *J. Pharmacol. Exp. Ther.*, 274(3): 1182–1191.
- Parsons, L.H., Smith, A.D. and Justice, J.B., Jr. (1991) Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. *Synapse*, 9(1): 60–65.
- Pennartz, C.M., Groenewegen, H.J. and Lopes Da Silva, F.H. (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. *Prog. Neurobiol.*, 42(6): 719–761.
- Pitt, B.R., Weng, W., Steve, A.R., Blakely, R.D., Reynolds, I. and Davies, P. (1994) Serotonin increases DNA synthesis in rat proximal and distal pulmonary vascular smooth muscle cells in culture. *Am. J. Physiol.*, 266(2): L178–L186.
- Porter, R.H., Benwell, K.R., Lamb, H., Malcolm, C.S., Allen, N.H., Revell, D.F., Adams, D.R. and Sheardown, M.J. (1999) Functional characterization of agonists at recombinant human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells. *Br. J. Pharmacol.*, 128(1): 13–20.
- Rea, W.P., Rothman, R.B. and Shippenberg, T.S. (1998) Evaluation of the conditioned reinforcing effects of phentermine and fenfluramine in the rat: concordance with clinical studies. *Synapse*, 30(1): 107–111.
- Roberts, D.C., Phelan, R., Hodges, L.M., Hodges, M.M., Bennett, B., Childers, S. and Davies, H. (1999) Self-administration of cocaine analogs by rats. *Psychopharmacology*, 144(4): 389–397.
- Rollema, H., Coe, J.W., Chambers, L.K., Hurst, R.S., Stahl, S.M. and Williams, K.E. (2007) Rationale, pharmacology and clinical efficacy of partial agonists of $\alpha_4\beta_2$ nACh receptors for smoking cessation. *Trends Pharmacol. Sci.*, 28(7): 316–325.
- Rose, S., Hunt, S., Collins, P., Hindmarsh, J.G. and Jenner, P. (1996) Repeated administration of escalating high doses of dexfenfluramine does not produce morphological evidence for neurotoxicity in the cortex of rats. *Neurodegeneration*, 5(2): 145–152.
- Rossetti, Z.L., Hmaidan, Y. and Gessa, G.L. (1992) Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *Eur. J. Pharmacol.*, 221(2–3): 227–234.
- Roth, B.L. (2007) Drugs and valvular heart disease. *N. Engl. J. Med.*, 356(1): 6–9.
- Rothman, R.B. and Baumann, M.H. (2008) Appetite suppressants, cardiac valve disease and combination pharmacotherapy. *Am. J. Ther.*, in press.
- Rothman, R.B., Aystas, M.A., Dersch, C.M. and Baumann, M.H. (1999) Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates: implications for primary pulmonary hypertension. *Circulation*, 100(8): 869–875.
- Rothman, R.B. and Baumann, M.H. (2002) Therapeutic and adverse actions of serotonin transporter substrates. *Pharmacol. Ther.*, 95(1): 73–88.
- Rothman, R.B. and Baumann, M.H. (2003) Monoamine transporters and psychostimulant drugs. *Eur. J. Pharmacol.*, 479(1–3): 23–40.
- Rothman, R.B. and Baumann, M.H. (2006) Therapeutic potential of monoamine transporter substrates. *Curr. Top. Med. Chem.*, 6(17): 1845–1859.
- Rothman, R.B., Baumann, M.H., Dersch, C.M., Romero, D.V., Rice, K.C., Carroll, F.I. and Partilla, J.S. (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*, 39(1): 32–41.
- Rothman, R.B., Baumann, M.H., Savage, J.E., Rauser, L., McBride, A., Hufseisen, S. and Roth, B.L. (2000a) Evidence for possible involvement of 5-HT_{2B} receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*, 102(23): 2836–2841.
- Rothman, R.B., Redmon, J.B., Raatz, S.K., Kwong, C.A., Swanson, J.E. and Bantle, J.P. (2000b) Chronic treatment with phentermine combined with fenfluramine lowers plasma serotonin. *Am. J. Cardiol.*, 85(7): 913–915.
- Rothman, R.B., Blough, B.E. and Baumann, M.H. (2002) Appetite suppressants as agonist substitution therapies for stimulant dependence. *Ann. N. Y. Acad. Sci.*, 965: 109–126.
- Rothman, R.B., Blough, B.E. and Baumann, M.H. (2007) Dual dopamine/serotonin releasers as potential medications for stimulant and alcohol addictions. *AAPS J.*, 9(1): E1–E10.
- Rothman, R.B., Blough, B.E., Woolverton, W.L., Anderson, K.G., Negus, S.S., Mello, N.K., Roth, B.L. and Baumann, M.H. (2005) Development of a rationally designed, low abuse potential, biogenic amine releaser that suppresses cocaine self-administration. *J. Pharmacol. Exp. Ther.*, 313(3): 1361–1369.
- Rothman, R.B., Clark, R.D., Partilla, J.S. and Baumann, M.H. (2003a) (+)-Fenfluramine and its major metabolite, (+)-norfenfluramine, are potent substrates for norepinephrine transporters. *J. Pharmacol. Exp. Ther.*, 305(3): 1191–1199.
- Rothman, R.B., Jayanthi, S., Wang, X., Dersch, C.M., Cadet, J.L., Prisinzano, T., Rice, K.C. and Baumann, M.H. (2003b) High-dose fenfluramine administration decreases serotonin transporter binding, but not serotonin transporter protein levels, in rat forebrain. *Synapse*, 50(3): 233–239.
- Rothman, R.B., Elmer, G.I., Shippenberg, T.S., Rea, W. and Baumann, M.H. (1998) Phentermine and fenfluramine: preclinical studies in animal models of cocaine addiction. *Ann. N. Y. Acad. Sci.*, 844: 59–74.
- Rothman, R.B., Gendron, T.M. and Hitzig, P. (1994) Combined use of fenfluramine and phentermine in the treatment of cocaine addiction: a pilot case series. *J. Subst. Abuse Treat.*, 11(3): 273–275.
- Rudnick, G. (1997) Mechanisms of biogenic amine transporters. In: Reith M.E.A. (Ed.), *Neurotransmitter Transporters: Structure, Function and Regulation*. Humana Press, Totowa NJ, pp. 73–100.
- Sachdev, M., Miller, W.C., Ryan, T. and Jollis, J.G. (2002) Effect of fenfluramine-derivative diet pills on cardiac valves: a meta-analysis of observational studies. *Am. Heart J.*, 144(6): 1065–1073.
- Setola, V., Hufeisen, S.J., Grande-Allen, K.J., Vesely, I., Glennon, R.A., Blough, B., Rothman, R.B. and Roth, B.L. (2003) 3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”) induces fenfluramine-like proliferative actions on

- human cardiac valvular interstitial cells in vitro. *Mol. Pharmacol.*, 63(6): 1223–1229.
- Setola, V. and Roth, B.L. (2005) Screening the receptorome reveals molecular targets responsible for drug-induced side effects: focus on 'fen-phen'. *Expert Opin. Drug Metab. Toxicol.*, 1(3): 377–387.
- Seuwen, K., Magnaldo, I. and Pouyssegur, J. (1988) Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT_{1B} receptors coupled to a Gi-protein. *Nature*, 335(Sept. 15): 254–256.
- Shearer, J., Wodak, A., Van Beek, I., Mattick, R.P. and Lewis, J. (2003) Pilot randomized double blind placebo-controlled study of dexamphetamine for cocaine dependence. *Addiction*, 98(8): 1137–1141.
- Sitte, H.H. and Freissmuth, M. (2003) Oligomer formation by Na⁺-Cl⁻-coupled neurotransmitter transporters. *Eur. J. Pharmacol.*, 479(1–3): 229–236.
- Smith, F.L., Yu, D.S., Smith, D.G., Leccese, A.P. and Lyness, W.H. (1986) Dietary tryptophan supplements attenuate amphetamine self-administration in the rat. *Pharmacol. Biochem. Behav.*, 25(4): 849–855.
- Steinbusch, H.W. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4): 557–618.
- Steranka, L.R. and Sanders-Bush, E. (1979) Long-term effects of fenfluramine on central serotonergic mechanisms. *Neuropharmacology*, 18(11): 895–903.
- Sulzer, D., Sonders, M.S., Poulsen, N.W. and Galli, A. (2005) Mechanisms of neurotransmitter release by amphetamines: a review. *Prog. Neurobiol.*, 75(6): 406–433.
- Torres, G.E. and Amara, S.G. (2007) Glutamate and monoamine transporters: new visions of form and function. *Curr. Opin. Neurobiol.*, 17(3): 304–312.
- Uhl, G.R. and Johnson, P.S. (1994) Neurotransmitter transporters: three important gene families for neuronal function. *J. Exp. Biol.*, 196(1): 229–236.
- Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand. Suppl.*, 367: 1–48.
- Vickers, S.P., Clifton, P.G., Dourish, C.T. and Tecott, L.H. (1999) Reduced satiating effect of D-fenfluramine in serotonin 5-HT_{2C} receptor mutant mice. *Psychopharmacology*, 143(3): 309–314.
- Volkow, N.D., Fowler, J.S. and Wang, G.J. (2002) Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. *Behav. Pharmacol.*, 13(5–6): 355–366.
- Volkow, N.D. and Li, T.K. (2004) Drug addiction: the neurobiology of behaviour gone awry. *Nat. Rev. Neurosci.*, 5(12): 963–970.
- Volkow, N.D., Wang, G.J., Fowler, J.S., Logan, J., Gatley, S.J., Hitzemann, R., Chen, A.D., Dewey, S.L. and Pappas, N. (1997) Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects. *Nature*, 386(Apr. 24): 830–833.
- Walsh, S.L., Haberny, K.A. and Bigelow, G.E. (2000) Modulation of intravenous cocaine effects by chronic oral cocaine in humans. *Psychopharmacology*, 150(4): 361–373.
- Wee, S., Anderson, K.G., Baumann, M.H., Rothman, R.B., Blough, B.E. and Woolverton, W.L. (2005) Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. *J. Pharmacol. Exp. Ther.*, 313(2): 848–854.
- Weiss, F., Parsons, L.H., Schulteis, G., Hyttia, P., Lorang, M.T., Bloom, F.E. and Koob, G.F. (1996) Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J. Neurosci.*, 16(10): 3474–3485.
- White, J.M. and Lopatko, O.V. (2007) Opioid maintenance: a comparative review of pharmacological strategies. *Expert Opin. Pharmacother.*, 8(1): 1–11.
- Wojnicki, F.H.E., Rothman, R.B., Rice, K.C. and Glowa, J.R. (1999) Effects of phentermine on responding maintained under multiple fixed-ratio schedules of food-presentation and cocaine-delivery in the rhesus monkey. *J. Pharmacol. Exp. Ther.*, 288(2): 550–560.
- Yu, Y.L., Fisher, H., Sekowski, A. and Wagner, G.C. (1997) Amphetamine and fenfluramine suppress ethanol intake in ethanol-dependent rats. *Alcohol*, 14(1): 45–48.
- Zaczek, R., Battaglia, G., Culp, S., Appel, N.M., Contrera, J.F. and De Souza, E.B. (1990) Effects of repeated fenfluramine administration on indices of monoamine function in rat brain: pharmacokinetic, dose response, regional specificity and time course data. *J. Pharmacol. Exp. Ther.*, 253(1): 104–112.
- Zolkowska, D., Baumann, M.H. and Rothman, R.B. (2008) Chronic fenfluramine administration increases plasma serotonin (5-HT) to non-toxic levels. *J. Pharmacol. Exp. Ther.*, 324(2): 791–797.
- Zolkowska, D., Rothman, R.B. and Baumann, M.H. (2006) Amphetamine analogs increase plasma serotonin: implications for cardiac and pulmonary disease. *J. Pharmacol. Exp. Ther.*, 318(2): 604–610.

CHAPTER 20

PTEN-5-HT_{2C} coupling: a new target for treating drug addiction

Jean-Christian Maillet*, Yun Zhang, Xuesheng Li and Xia Zhang

University of Ottawa Institute of Mental Health Research, Ottawa, Ont., Canada K1Z 7K4

Abstract: It is well known that the ventral tegmental area (VTA) is a brain region in which virtually all abused drugs exert rewarding effects by activating its dopamine neurons. We recently found that the tumour suppressor enzyme phosphatase and tensin homologue deleted on chromosome 10 (PTEN) directly interacts to a region in the third intracellular loop (3L4F) of serotonin 5-HT_{2C} receptors (5-HT_{2C}R) in the rat VTA. PTEN limits agonist-induced 5-HT_{2C}R phosphorylation via its protein phosphatase activity. Systemic or intra-amygdaloid application of the interfering peptide Tat-3L4F is able to disrupt PTEN coupling with 5-HT_{2C}R in the rat VTA, resulting both in a suppression of the increased firing rate of VTA dopaminergic neurons induced by Δ^9 -tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, and in a blockade of the conditioned place preference induced by THC and nicotine [Ji, S.P. et al. (2006). *Nat. Med.*, 12: 324–329]. Because the blockade effects of Tat-3L4F peptide on the conditioned preference could be achieved by the suppression of Tat-3L4F peptide on the rewarding and/or learning/memory mechanisms associated with conditioned place preference, we recently explored whether Tat-3L4F can affect learning and memory. We observed that Tat-3L4F did not produce significant effects on spatial learning and memory in a Morris water maze test, thus indicating that Tat-3L4F can effectively suppress the rewarding effects induced by drugs of abuse.

Keywords: Tat-3L4F; drug addiction; G-protein; coupled receptors; 5-HT_{2C} receptor; PTEN; cannabinoids; ventral tegmental area

Introduction

Drugs of abuse have distinct pharmacological properties, each acting on their respective receptors in the brain and in the periphery to elicit distinct behavioural and physiological effects upon administration. However, one common aspect of drugs of abuse is their rewarding properties, which lead to

increased consumption and eventually addiction. Drug addiction had been traditionally viewed as the motivation of an addict to use drugs results from the desire to experience the rewarding (e.g. hedonic) effects of the drug as well as from the desire to avoid the punishing (e.g. anhedonic or aversive) consequences of drug withdrawal. Intensive studies during the past two decades have added new knowledge about the mechanisms of drug addiction. The current understanding of drug addiction is that repeated drug use abnormally stimulates neurons responding to natural reinforcers such as

*Corresponding author. Tel.: +(613) 722-6521, Ext. 6043; Fax: +(613) 761-3610; E-mail: Jean-Christian.Maillet@rohcg.on.ca

food and sex, leading to long-lasting adaptation changes of brain reward pathways (Koob and Le Moal, 1997; Nestler, 2004) and aberrant learning processes (Robbins and Everitt, 1999, 2002; Self et al., 2004; Liu et al., 2005; Wang et al., 2007). Drug addiction has many faces, including initiation or euphoria phase (i.e. drug-induced rewarding or reinforcing effects), maintenance or dependence phase (i.e. compulsive drug taking), tolerance, withdrawal episodes, protracted abstinence and craving and relapse (or reinstatement). Animal studies on drug addiction have paid special attention to elucidating the mechanisms underlying drug-induced rewarding effects, drug dependence (characterized by withdrawal syndrome) and craving or relapse of drug seeking.

The ventral tegmental area (VTA) is a midbrain region that has been implicated in the rewarding motivational effects of a wide variety of addictive drugs including cannabinoids and nicotine (for review, see Gardner, 2005; Laviolette and van der Kooy, 2005). Within the VTA there is a population of dopamine (DA) neurons that send ascending projections to the nucleus accumbens (NAc) and the prefrontal cortex, which serves as a common signalling pathway that underlies the rewarding effect of drugs of abuse, regardless of their individual mechanism of action.

The serotonin neurotransmitter system exerts a modulatory effect on the VTA. Application of 5-hydroxy-tryptamine (5-HT) or stimulation of the raphe nuclei that send serotonergic innervation to the VTA elicits changes in the firing rate of VTA DA neurons, likely via the stimulation of multiple 5-HT receptor subtypes (Cameron and Williams, 1994; Gervais and Rouillard, 2000). In relation to regulation of VTA dopaminergic neuronal activity, particular attention has been paid to the 5-HT_{2c} receptor. Studies have shown that 5-HT_{2c} mRNA (Eberle-Wang et al., 1997) and protein (Bubar and Cunningham, 2007) are expressed in the VTA. Pharmacological blockade of the 5-HT_{2c} receptor resulted in an increase in the basal firing of DA neurons in the VTA, and microdialysis studies revealed an increased release of DA in the NAc (Di Giovanni et al., 1999; Di Matteo et al., 2000; De Deurwaerdere et al., 2004). In follow-up experiments it was found that treatment with the 5-HT_{2c}

receptor agonist Ro600175 caused a decrease in the basal firing of VTA DA neurons, an effect that was blocked by the antagonist SB 242084 (Di Matteo et al., 2000). The results from these experiments suggest that the 5-HT_{2c} receptors exert inhibitory control on the VTA.

As indicated above, drugs of abuse appear to produce rewarding effects by acting within the VTA itself to increase DA neuron activity, leading to the enhancement of extracellular DA concentrations in the NAc. Therefore, the inhibitory control of the 5-HT_{2c} receptors on the VTA dopaminergic neurons implies the involvement of 5-HT_{2c} receptors in drug addiction. Indeed, evidence from pharmacological studies suggests that 5-HT_{2c} receptors could modulate dopaminergic signalling in the VTA in response to drugs of abuse. Both systemic and intra-VTA administration of Ro600175 significantly decreased hyperlocomotive activity and the self-administration of drugs such as nicotine and cocaine (Grottick et al., 2001; Fletcher et al., 2004; Pierucci et al., 2004). These effects were all blocked by the 5-HT_{2c} antagonist SB 242084. Grottick et al. (2001) went on to demonstrate that administration of Ro600175 also blocked the self-administration of food in an operant conditioning paradigm, which clearly demonstrates that the activity of the 5-HT_{2c} receptors blunts the effects of the VTA–NAc reward pathway in the brain.

Activation of the 5-HT_{2c} receptors by serotonin or agonists promotes receptor phosphorylation (Westphal et al., 1995; Backstrom et al., 2000). The phosphorylation of G-protein coupled receptors (GPCRs) such as 5-HT_{2c} receptor is a common mechanism for desensitization and resensitization of receptors after ligand activation, which is crucial for efficient signalling by the receptor (for review, see Gainetdinov et al., 2004). Backstrom et al. (2000) demonstrated that phosphorylation of the 5-HT_{2c} receptor was required for efficient signalling. Deleting the last three amino acids at the carboxyl terminal of 5-HT_{2c} receptor abrogated serotonin-mediated phosphorylation of the receptor, resulting in decreased activity. These results suggest that phosphorylation of the 5-HT_{2c} receptor is required for its activity. Based on these results it is reasonable to hypothesize that regulation of the phosphorylation state of the 5-HT_{2c}

receptors could also regulate its ability to modulate the activity of VTA DA neurons.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a widely expressed protein with dual lipid and protein phosphatase activity, is a well characterized tumour suppressor that impinges on the mitogenic phosphoinositol-3 kinase pathway (for review, see [Leslie and Downes, 2004](#)). Recent studies demonstrate that PTEN is widely expressed in the brain where it plays a role in neuronal development ([Lachyankar et al., 2000](#)) and regulation of extrasynaptic signalling via protein–protein interaction ([Ning et al., 2004](#)). Given its widespread expression in the adult brain and its ability to regulate the phosphorylation state of proteins, it is possible that PTEN may regulate various signalling pathways such as the VTA dopaminergic pathway via protein–protein interactions with receptors such as the 5-HT_{2c} receptor.

PTEN associates directly with the 5-HT_{2c} receptor

To test the initial hypothesis that there is an interaction between PTEN and the 5-HT_{2c} receptor, immunoprecipitations were performed on cultured cells. Primary antibodies directed towards PTEN were able to precipitate endogenous 5-HT_{2c} receptor and vice versa, suggesting that PTEN and the 5-HT_{2c} receptor were part of the same protein complex ([Fig. 1a](#)).

In order to map the interaction between PTEN and the 5-HT_{2c} receptor, the full length 5-HT_{2c} receptor was divided into fragments to determine the region of the receptor that mediated the interaction with PTEN. We initially hypothesized that the C-terminal of the 5-HT_{2c} receptor contains a protein sequence coupling to PTEN, because ample evidence showed that the C-terminal of a GPCR usually contains protein residues interacting directly with other receptors or other intracellular proteins ([Lee et al., 2002, 2003](#)). To test this hypothesis, we constructed a fusion protein containing the full sequence of the C-terminal of the 5-HT_{2c} receptor and then used it in a pull down assay. However, we tried again and again without being able to find positive evidence suggesting the interaction of the C-terminal of the

5-HT_{2c} receptor with PTEN. After a few weeks, it suddenly came to our attention that the third intracellular loop of the 5-HT_{2c} receptor is almost as long as its C-terminal ([Fig. 2](#)), suggesting the possibility that the third intracellular loop of the 5-HT_{2c} receptor, but not its C-terminal, may contain the protein residue responsible for its interaction with PTEN. This hypothesis is supported by our subsequent pull down experiment employing two fusion proteins containing the full sequences of the C-terminal and third intracellular loop of the 5-HT_{2c} receptor, respectively ([Fig. 1b](#)).

Next, we tried to answer the question of which part of the third intracellular loop of the 5-HT_{2c} receptor mediated its physical association with PTEN. The third intracellular loop was further divided into five fragments: 3L1F (Leu237–Gly252), 3L2F (His253–Asn267), 3L3F (Cys268–Asn282), 3L4F (Pro283–Arg297) and 3L5F (Pro298–Lys313). Five corresponding fusion proteins were constructed and then used in pull down assays to determine if PTEN is directly associated with the 5-HT_{2c} receptor. Results from these experiments allowed us to determine that the 3L4F fragment mediated the direction protein–protein coupling of the 5-HT_{2c} receptor and PTEN ([Fig. 1c, d](#)).

In order to further confirm whether 3L4F is critical for the protein–protein interaction between the 5-HT_{2c} receptor and PTEN, we explored whether application of 3L4F peptide to cell lysate before immunoprecipitation could block immunoprecipitation of 5-HT_{2c} receptor with PTEN and vice versa. As shown in [Fig. 1e](#), we observed that pretreatment of 3L4F peptide blocked immunoprecipitation between the 5-HT_{2c} receptor and PTEN. These results not only suggest the importance of 3L4F in the protein–protein interaction between the 5-HT_{2c} receptor and PTEN, but also implying that 3L4F peptide could be used for disruption of the protein–protein coupling between the 5-HT_{2c} receptor and PTEN.

Given the ability of PTEN to dephosphorylate proteins via its protein phosphatase activity, we sought to determine if PTEN could regulate phosphorylation of the 5-HT_{2c} receptor. To test this hypothesis we prepared stable cell lines where PTEN expression was knocked down using small interfering RNA (siRNA) strategy or where

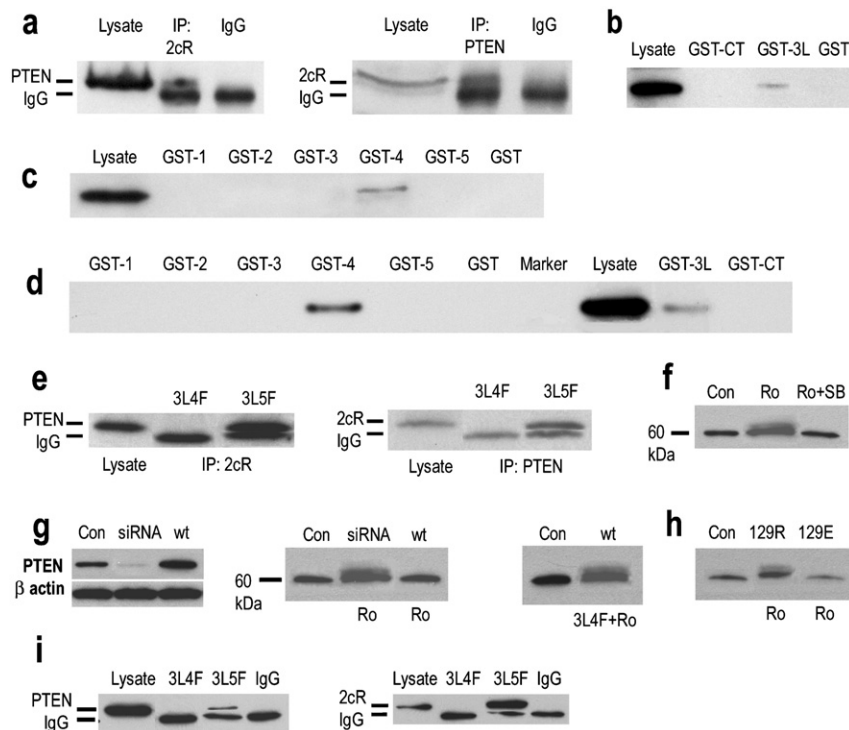


Fig. 1. Interaction of PTEN with 5-HT_{2c} receptor (5-HT_{2c}R) in PC12 cells. (a) Co-immunoprecipitation of PTEN by anti-5-HT_{2c}R antibody (left) and co-immunoprecipitation of 5-HT_{2c}R by anti-PTEN antibody (right). (b) Western blot of PC12 PTEN after affinity precipitation by GST-loop 3 (GST-3L), but not by GST-C terminal (GST-CT) or GST alone. (c) Western blot of PC12 PTEN after affinity precipitation by GST-fragment 4 (GST-4), but not by GST-1, GST-2, GST-3, GST-5 or GST alone. (d) Direct binding of purified PTEN with GST-4 or GST-3L, but not with GST-1, GST-2, GST-3, GST-5, GST alone or GST-CT. (e) Application of 3L4F, but not 3L5F, into cell lysates before co-immunoprecipitation blocked immunoprecipitation of PTEN by anti-5-HT_{2c}R antibody (left) or 5-HT_{2c}R by anti-PTEN antibody (right). (f) Anti-5-HT_{2c}R antibody detected a 60-kDa protein band in PC12 cells without treatment (Con) or after treatment with both Ro600175 and SB242084 (Ro + SB), whereas an additional 61-kDa protein band was revealed after Ro600175 treatment (Ro). (g) Left: Western blot showed decreased or increased PTEN in PC12 cells over-expressing siRNA PTEN (siRNA) or wild-type PTEN (wt) relative to control PC12 cells (Con), without detectable changes in β -actin levels (bottom). Middle: Relative to Con, the 61-kDa protein band appeared in G129R-overexpressed PC12 cells (129R) but not in G129E-overexpressed cells (129E) following Ro600175 treatment (Ro). Right: Relative to Con, wt stable cells showed a 61-kDa protein band after co-treatment with Tat-3L4F (3L4F) and Ro600175 (Ro). (h) Ro600175 (Ro) induced a 61-kDa protein band in siRNA PTEN stable cells (siRNA) but not in wild-type PTEN stable cells (wt) relative to control cells (Con). (i) Application of 3L4F, but not 3L5F, into samples dissected from the VTA blocked co-immunoprecipitation of PTEN by anti-5-HT_{2c}R antibody (left) or 5-HT_{2c}R by anti-PTEN antibody (right).

wild-type PTEN was over-expressed. The 5-HT_{2c} receptor agonist Ro600175 induced phosphorylation of the 5-HT_{2c} receptor in cells in which PTEN expression had been knocked down, but not in cells over-expressing PTEN (Fig. 1f, g), suggesting that PTEN can dephosphorylate the 5-HT_{2c} receptor.

PTEN contains both lipid phosphatase, which can negatively regulate the serine/threonine kinase Akt to exert its tumour suppressor function

(Maehama and Dixon, 1998; Stambolic et al., 1998), and protein phosphatase, which has been shown to dephosphorylate phosphotyrosine- and phosphoserine/threonine-containing substrates (Myers et al., 1997). To further determine the role of protein phosphatase and lipid phosphatase of PTEN in regulating phosphorylation of the 5-HT_{2c} receptor, we prepared additional stable PC12 cell lines that over-expressed either of two

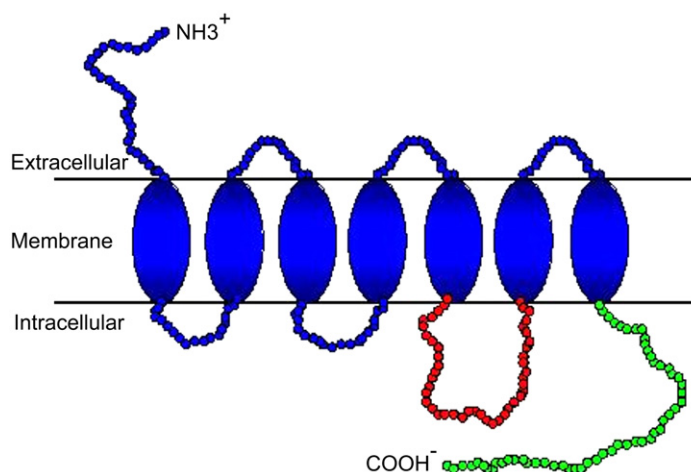


Fig. 2. Illustration of the structure of the 5-HT_{2c} receptor. The third intracellular loop (red) with 76 amino acids is shorter than the C-terminal (green) with 89 amino acids. (See Color Plate 20.2 in color plate section.)

dominant-negative forms of PTEN, G129R or G129E. G129R encodes PTEN without both protein and lipid phosphatase activities (Furnari et al., 1997), whereas G129E causes loss of lipid phosphatase activity with retention of protein phosphatase activity (Weng et al., 2001). We observed that Ro600175 produced formation of the 61-kDa protein in G129R-over-expressed PC12 cells but not in G129E-over-expressed cells (Fig. 1h), indicating that 5-HT_{2c}R phosphorylation is regulated by protein phosphatase of PTEN but not by its lipid phosphatase. These results suggest that the functional protein–protein interaction of PTEN to the 5-HT_{2c} receptor is achieved via control of the 5-HT_{2c} receptor by protein phosphatase of PTEN.

Overall, the implications of our *in vitro* results are that protein–protein coupling of PTEN with the 5-HT_{2c} receptor could modulate responses induced by drugs of abuse by likely regulating the phosphorylation state of 5-HT_{2c} receptor. To test this hypothesis it is important to establish that the interaction between PTEN and the 5-HT_{2c} receptor occurs *in vivo*. Co-immunoprecipitation analysis from lysates obtained from the VTA of rats demonstrated that PTEN and the 5-HT_{2c} form a complex in this brain area. Furthermore when adding the 3L4F peptide to samples before immunoprecipitation prevented the association of

PTEN with the 5-HT_{2c} receptor (Fig. 1i). Thus, these results suggest that a PTEN–5-HT_{2c} receptor complex exists in the VTA, which could be disrupted by 3L4F peptide.

It has been shown that activation and inactivation of the 5-HT_{2c} receptor produced by its agonist and antagonist, respectively, reduces and increases the firing rate of VTA DA neurons innervating the NAc (Di Matteo et al., 2002; Higgins and Fletcher, 2003). In order to convincingly demonstrate that protein–protein coupling of PTEN with the 5-HT_{2c} receptor could modulate responses induced by drugs of abuse, we conducted *in vivo* electrophysiological experiments to explore how PTEN–5-HT_{2c} receptor complex in the rat VTA regulates neuronal activity of VTA dopaminergic neurons that selectively innervate neurons in the NAc. To investigate the function of PTEN–5-HT_{2c} receptor complex *in vivo*, 3L4F peptide has to be used for disrupting this complex. We rendered the 3L4F motif cell permeable by fusing it to the Tat peptide motif so as to give 3L4F peptide such a capacity of penetrating through the blood–brain barrier after a systemic injection (Schwarze et al., 1999). We observed that systemic and intra-VTA injections of the Tat-3L4F peptide significantly suppressed the firing rates of VTA dopaminergic neurons, an effect that was blocked by the 5-HT_{2c} receptor antagonist SB242084 (Fig. 3a). These results

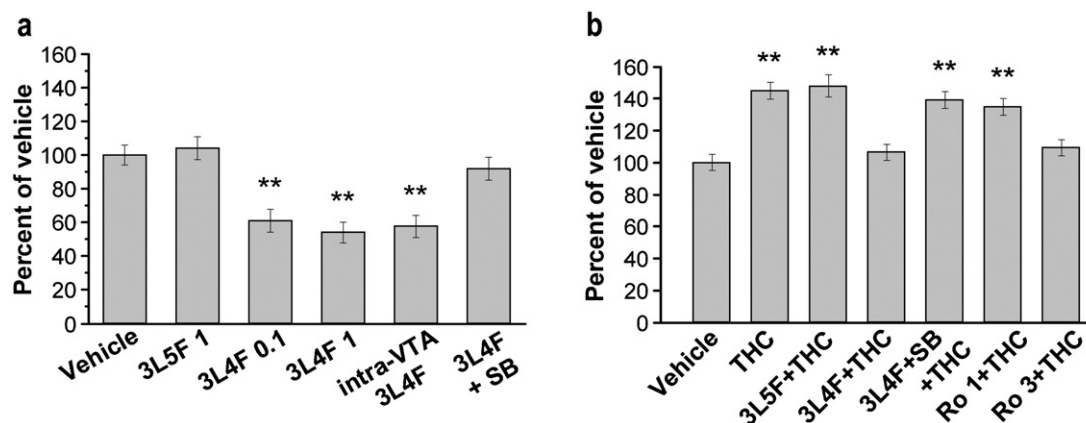


Fig. 3. Effects of 5-HT_{2c}R modulation and THC on the firing rate of VTA dopamine neurons. Antidromic stimulation of the shell of the NAc was used to identify VTA–NAc neuron, followed by recording of the firing rate of the VTA dopamine neuron. (a) Relative firing rate of VTA dopamine neurons following an i.v. administration of Tat-3L5F (3L5F) and Tat-3L4F (3L4F) ($n = 5$). There was a significant overall difference (one-way ANOVA: $F_{5,24} = 26.469$, $p < 0.01$). While Tat-3L5F (1 $\mu\text{mol/kg}$, i.v.) produced no significant effects, both i.v. injection of Tat-3L4F (1 and 0.1 $\mu\text{mol/kg}$) and intra-VTA application of Tat-3L4F (10 nmol) induced similar inhibitory effects on the firing rate of VTA dopamine neurons ($p < 0.01$). The inhibitory effects of Tat-3L4F (0.1 $\mu\text{mol/kg}$, i.v.) were temporarily abolished by SB242084 (SB, 0.6 mg/kg, i.v.). (b) Relative firing rate of VTA dopamine neurons following an i.v. administration of THC and pretreatments with Tat-3L5F (3L5F), Tat-3L4F (3L4F), Ro600175 (Ro) and SB242084 (SB) ($n = 5$). One-way ANOVA showed a significant overall difference ($F_{6,28} = 14.986$, $p < 0.01$). Tukey *post hoc* test further revealed that THC (0.3 mg/kg) significantly increased the firing rate ($p < 0.01$), which was reversed by Tat-3L4F (0.1 $\mu\text{mol/kg}$) or the high dose of Ro600175 (3 mg/kg), but not by Tat-3L5F (0.1 $\mu\text{mol/kg}$) or the low dose of Ro600175 (1 mg/kg). The blockade effects of Tat-3L4F (0.1 $\mu\text{mol/kg}$) on THC-induced increased firing rate were temporarily abolished by SB242084 (0.6 mg/kg). * $p < 0.05$, ** $p < 0.01$ as compared with vehicle.

therefore suggest that indeed, the uncoupling of the 5-HT_{2c} receptor from PTEN mimics the effects of receptor activation. In a follow-up experiment Δ^9 -tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana or cannabis, was administered either alone or co-administered with the Tat-3L4F to determine if the interfering peptide could block THC-induced increase of VTA neuronal firing. Results showed that indeed the Tat-3L4F peptide was able to suppress the increase in VTA dopaminergic neuronal firing induced by THC (Fig. 3b). These data suggest that disrupting the interaction between PTEN and the 5-HT_{2c} receptor could suppress the rewarding effect of marijuana, given that THC and the more potent and efficacious cannabinoid receptor agonists WIN55,212-2, HU210 and CP55940 significantly increased neuronal firing rate in anaesthetized and un-anaesthetized rats (French et al., 1997; Gessa et al., 1998; Wu and French, 2000), as well as in brain slices containing the VTA (Cheer et al., 2000).

Uncoupling of PTEN to 5-HT_{2c} receptor suppresses rewarding effects of drugs of abuse

The rewarding effect of drugs of abuse is correlated with an increase in firing of VTA dopaminergic neurons, an effect that was blocked by uncoupling PTEN from the 5-HT_{2c} receptor. These results raised the possibility that the Tat-3L4F peptide could suppress the behaviours associated with the rewarding effect of drugs of abuse. This hypothesis was first tested using a conditioned place preference (CPP) paradigm consisting of pretest, conditioning and test sessions. In the pretest and test sessions, rats were allowed to freely explore two distinct compartments; time spent in each compartment was recorded. During conditioning sessions, THC, the psychoactive ingredient of marijuana, and vehicle were paired with different compartments. The difference between time spent in the drug-paired vs. vehicle-paired compartment during pretest and

test sessions was then compared. During conditioning sessions, Tat-3L4F interfering peptide used in the electrophysiological studies was injected 1 h before THC. Administration of the Tat-3L4F peptide blocked CPP in rats induced by THC or nicotine (Fig. 4). These results suggest that the uncoupling of PTEN from the 5-HT_{2c} receptor could suppress the rewarding properties of drugs of abuse.

Although the CPP paradigm measures rewarding effects, it is highly contingent on classical conditioning. This contingency raises the possibility that the ability of Tat-3L4F peptide to suppress the rewarding properties of cannabis and nicotine stem from defects in learning and memory rather than an impingement on the reward pathway. This idea is supported by two lines of indirect evidence. Firstly, a recent study has shown that PTEN knockout mice demonstrated disrupted

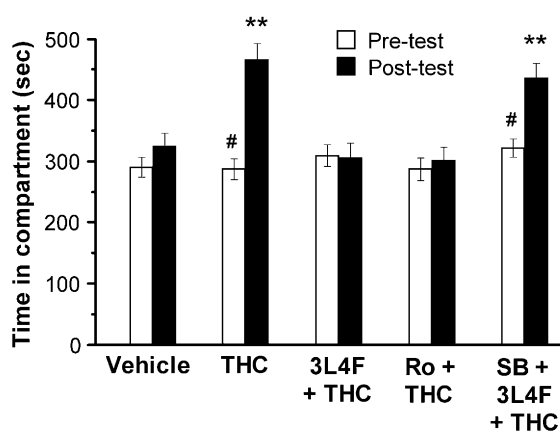


Fig. 4. Effects of Tat-3L4F and the 5-HT_{2c}R agonist Ro600175 on the conditioned place preference (CPP). Data were presented as means \pm SEM ($n = 8$ for each group). The CPP test was used to evaluate the rewarding effects of THC and various drug treatments by comparing the time spent in the conditioned compartment before and after conditioning, i.e. in pre- and post-test. Both THC (0.3 mg/kg, i.p.) and nicotine (1 mg/kg, i.p.) significantly increased the time spent in conditioned compartment ($p < 0.01$), i.e. CPP, whereas daily application of Tat-3L4F (3L4F, 0.1 μ mol/kg, i.p.) or Ro600175 (Ro, 3 mg/kg, i.p.) before THC or nicotine prevented the CPP induced by THC or nicotine. The suppression of THC-induced CPP by Tat-3L4F was prevented by pre-treatment with the 5-HT_{2c}R antagonist SB242084 (SB, 0.6 mg/kg, i.p.). One-way ANOVA showed significant overall difference ($F_{13,98} = 11.831$, $p < 0.01$).

long-term depression (LTD) in mouse hippocampal slices without significant effects on long-term potentiation (LTP) (Wang et al., 2006). It has long been known that both LTD and LTP participate in the synaptic plasticity that underlies the cellular mechanism of certain forms of learning and memory. Specifically, it has been shown that stress not only impaired hippocampus-dependent spatial memory (Wong et al., 2007), but also inhibited hippocampal LTP and enhanced LTD (Xu et al., 1997). We recently explored how coincident activity mediated through hippocampal converging afferent pathways affects the formation of hippocampal LTP and LTD in relation to spatial learning. We found that hippocampus-dependent spatial learning was severely impaired when hippocampal input activity for the induction of the bilateral LTP and LTD was suppressed before spatial learning task (unpublished observation). Therefore, it is plausible to hypothesize that disruption of hippocampal LTD in PTEN knockout mice suggests the possibility that disruption of PTEN interaction with the 5-HT_{2c} receptor may affect hippocampal-dependent spatial learning and memory, although all the available evidence so far does not allow us to safely exclude the possibility that disruption of LTD in PTEN knockout mice was due to a compensatory response during development.

Secondly, we have recently observed that down-regulating the protein expression of PTEN in hippocampal neurons inhibits the function of extrasynaptic *N*-methyl-D-aspartate receptor (NMDAR) and decreases NMDAR surface expression, suggesting a crucial role for endogenous PTEN in the modulation of NMDAR-mediated neuronal function (Ning et al., 2004). Because NMDA receptors are well documented to participate in learning and memory processes associated with drug addiction (Robbins and Everitt, 1999, 2002; Self et al., 2004; Liu et al., 2005; Wang et al., 2007), these findings also indicate that disruption of PTEN interaction with the 5-HT_{2c} receptor may affect hippocampal-dependent spatial learning and memory through NMDAR-mediated signalling pathway.

Based on the above considerations, we recently conducted experiments to specifically test whether

the Tat-3L4F affected hippocampal-dependent spatial learning and memory. In one experiment, rats were administered the cannabinoid HU210, Tat-3L4F or vehicle once per day for 4 days before four sessions of spatial learning in the Morris water maze paradigm. Vehicle-injected rats showed significant spatial learning during the four daily sessions of training consisting of four trials in each session (Fig. 5), indicating that our training protocol is sufficient to produce spatial learning. This idea is further supported by our findings that administration of HU210 prominently suppressed spatial learning, which is consistent with the results obtained from two independent groups (Ferrari et al., 1999; Hill et al., 2004). However, there was no significant difference in spatial learning between rats administered the Tat-3L4F peptide or vehicle (Fig. 5), suggesting that Tat-3L4F does not impair the ability of rats to learn (Li et al., submitted). In a second experiment, rats were trained to acquire spatial memories. One day after four training sessions the rats were treated with either Tat-3L4F or vehicle and then tested to determine if rats could retrieve the spatial memory. Administration of the Tat-3L4F did not significantly affect the retrieval of spatial memories (Fig. 6), suggesting that Tat-3L4F does not produce significant effects on the ability of rats

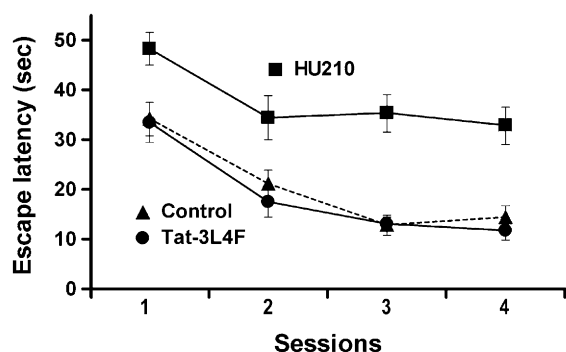


Fig. 5. Effects of HU210 and Tat-3L4F on the requisition of spatial memory. In a standard spatial learning version of the Morris water maze, rats were treated with vehicle, HU210 and Tat-3L4F 60 min before each of four consecutive daily sessions with each session consisting of four trials, during which the mean escape latency was recorded. HU210 (100 μ g/kg, i.p.), but not Tat-3L4F (1 μ mol/kg, i.p.), significantly increased the escape latency relative to vehicle injection.

to memorize spatial information (Li et al., submitted).

When taken together, the results of these behavioural tests suggest that disrupting the interaction between PTEN and the 5-HT_{2c} receptor with Tat-3L4F peptide is able to suppress the rewarding effects of drugs of abuse without affecting learning and memory processes.

Similarity and difference of Tat-3L4F and 5-HT_{2c} receptor agonist in inducing behavioural response

Treatment with the 5-HT_{2c} agonist Ro600175 and the Tat-3L4F peptide both resulted in a significant decrease in the firing rates of VTA neurons (Di Matteo et al., 2000; Ji et al., 2006). These results suggest that decreasing PTEN-mediated dephosphorylation of the 5-HT_{2c} receptor is functionally similar to an agonist-mediated increase in receptor phosphorylation. Furthermore, both treatment with Tat-3L4F peptide and Ro600175 blocked THC-induced CPP (Fig. 4). These results, along with previous results showing the generalized therapeutic effect of Ro600175 against the self-administration of drugs such as cocaine, ethanol and nicotine (Higgins and Fletcher, 2003), suggest a potential universal strategy for treating drug addiction with the Tat-3L4F peptide.

A potential concern for treating drug addiction with the Tat-3L4F peptide is the numerous side effects associated with the pharmacological intervention of the 5-HT_{2c} receptor. Treatment with the agonist Ro600175 is associated with behavioural changes such as penile erection, hypophagia, hypolocomotion, motor functional suppression and anxiety (Clifton et al., 2000; Grottick et al., 2000; Higgins and Fletcher, 2003; Wood, 2003; Alves et al., 2004). Given the similarities in the electrophysiological and behavioural responses between Ro600175 and the Tat-3L4F peptide, it is possible that treatment with the Tat-3L4F may induce similar side effects. To test this hypothesis, rats were treated with either Ro600175 or the Tat-3L4F peptide and subjected to a battery of behavioural tests. Ro600175, but not Tat-3L4F, induced penile erection (data not shown), anxiety (Fig. 7a, b), hypophagia (Fig. 7c),

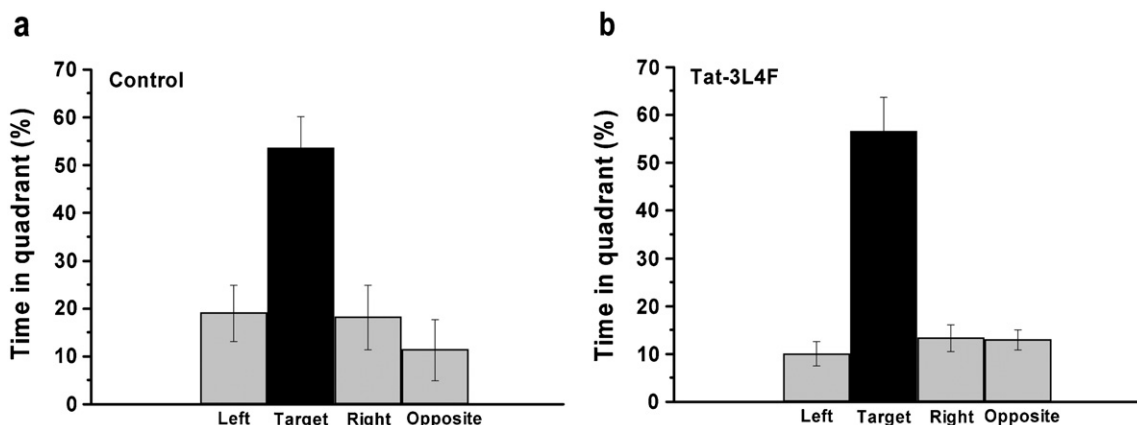


Fig. 6. Effects of Tat-3L4F on the retrieval of spatial memory. One day after four daily sessions of training with four trials in each session, rats were injected with vehicle or Tat-3L4F (1 μ mol/kg), followed 1 h later by a probe trial. Rats receiving either vehicle (a) or Tat-3L4F (1 μ mol/kg, i.p.) (b) before the probe trial showed similar preference to the target quadrant where the platform had been located.

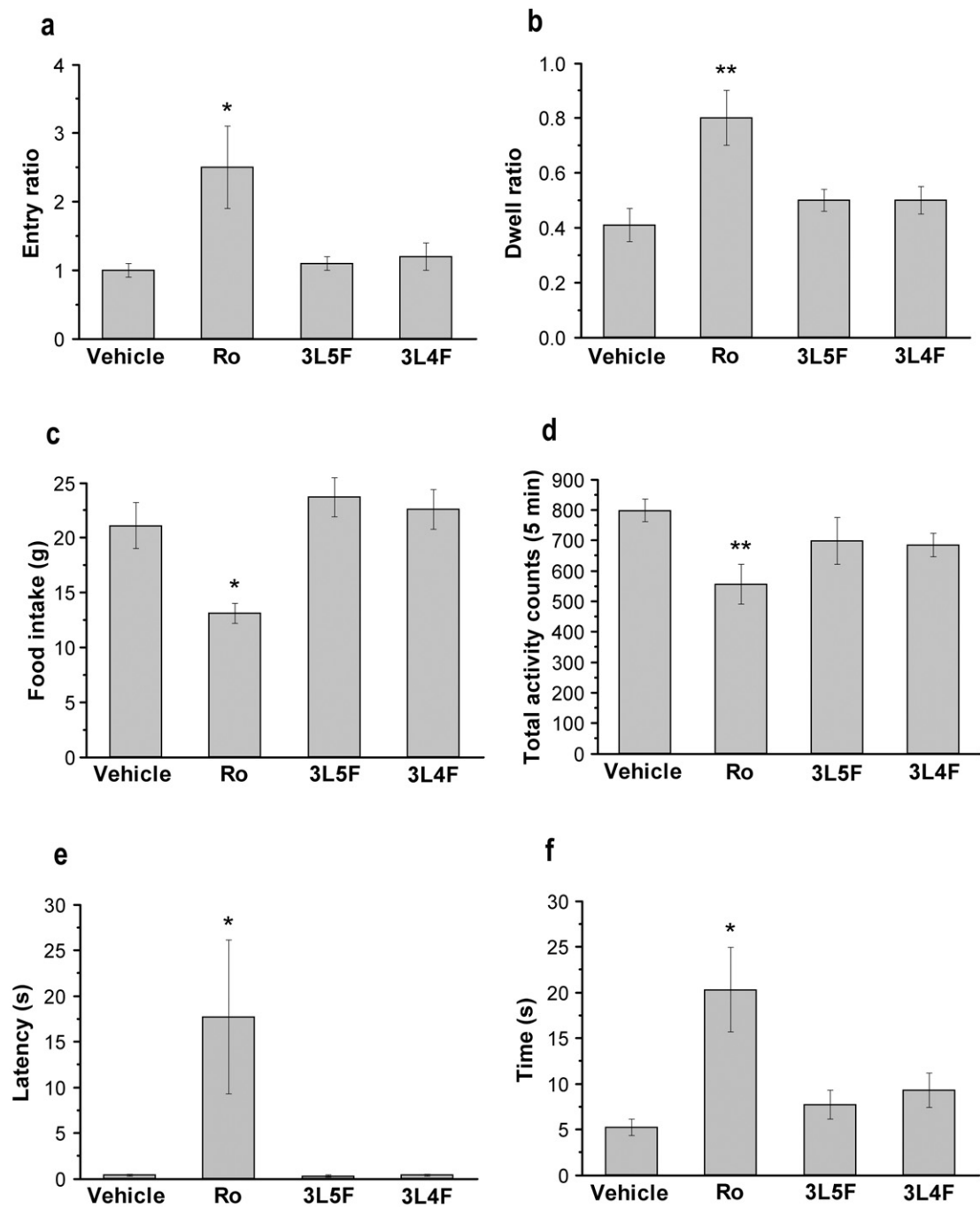
hypolocomotion (Fig. 7d) as well as motor functional suppression in incline grid (Fig. 7e) and elevated beam-walk tests (Fig. 7f) but not in the catalepsy bar test (data not shown). These data indicate that the Tat-3L4F peptide can suppress the rewarding effect of THC without the profound side effects observed with Ro600175 treatment. It is possible that PTEN-regulated phosphorylation of the 5-HT_{2c} receptors is crucial in regulating the rewarding effects of drugs, whereas different intracellular pathways may mediate Ro600175-induced behavioural changes.

Location of PTEN-5-HT_{2c}R complex in the VTA

Biochemical, electrophysiological and behavioural evidence suggests that uncoupling PTEN from the 5-HT_{2c} receptor suppresses physiological and behavioural responses to THC. Yet the exact neuroanatomical signalling pathway through which these effects are mediated is still unclear.

As described above, several studies suggest that the cannabinoid-induced increase in DA accumulation in the NAc may result from an increase in DA neuron firing and burst rates as a result of CB₁ receptor activation. Furthermore, the increased activity of VTA DA neurons caused by the synthetic cannabinoid HU210 in brain slices

(Cheer et al., 2000) implies that the cannabinoids must act either directly upon the DA neurons themselves, which is unlikely, given the absence of cannabinoid receptors on these neurons (Herkenham et al., 1991), or upon the local circuitry of the VTA to increase DA neuron activity. In fact, this study also reported that prior application of the GABA-A receptor antagonist, bicuculline, blocked the excitatory effect of HU210 (Cheer et al., 2000), suggesting that, like opioid receptors (Johnson and North, 1992), CB₁ receptors may increase VTA DA neuron activity via a local disinhibitory mechanism. This idea has recently gained more direct support with the finding that WIN55,212-2 application in brain slices containing the VTA could reduce electrically evoked inhibitory post-synaptic currents (IPSCs) mediated by GABA-A receptor activation (Szabo et al., 2002). Furthermore, this effect appeared to be mediated by CB₁ receptors located on the inhibitory terminals of GABAergic neurons intrinsic to the VTA, since it was blocked by the CB₁ antagonist SR141716A as well as by dendritic application of the GABA-A receptor agonist muscimol (Szabo et al., 2002). In addition, spontaneously occurring IPSCs that were resistant to tetrodotoxin were also unaffected by WIN55,212-2, further suggesting that this drug did not act postsynaptically to diminish GABAergic IPSCs. Since the presynaptic inhibition of



neurotransmitter release is one of the most frequently observed and best characterized effects of CB1 receptor activation in the brain (Hoffman and Lupica, 2000, 2001; Schlicker and Kathmann, 2001; Hoffman et al., 2003), and subpopulations of GABAergic terminals throughout the brain are densely populated by CB1 receptors (Freund et al., 2003), it is not surprising that this effect was observed.

Taking together, cannabinoids may increase activity of VTA DA neurons through a disinhibitory mechanism produced by a decrease in the release of GABA in the VTA. Based on these results our lab proposed a model whereby increased neurotransmission in the VTA–NAc DA pathway may occur via cannabinoid-induced decreased release of GABA in the VTA, in combination with a decrease in 5-HT2c phosphorylation in VTA neurons via coupling with PTEN. A decrease in receptor phosphorylation may delay resensitization of 5-HT2c responses, resulting in diminished signalling. As such, disruption of the interaction between PTEN and 5-HT2c receptor by the Tat-3L4F peptide may increase receptor phosphorylation and activation. Therefore, it is possible that an increase in 5-HT2c activity could counteract the effects of drug-induced decrease in GABA release in the VTA.

Evidence supporting this model stems from immunohistochemical experiments demonstrating that PTEN co-localizes with tyrosine hydroxylase (TH), a marker for DA neurons. Moreover, 90% of PTEN positive neurons co-stained with 5-HT2c receptors suggest that PTEN co-localizes in dopaminergic neurons (Ji et al., 2006). Results from our lab suggesting that 5-HT2c receptors are expressed in dopaminergic neurons have been since supported by immunohistochemical studies showing that the receptor co-localizes with TH in the rat VTA (Bubar and Cunningham, 2007).

Evidence from other labs argues that stimulation of the 5-HT2c receptor either by serotonin or agonist produces an excitatory response. A study by Stanford et al. (2005) reports excitatory responses from stimulated 5-HT2c receptors. These excitatory responses are explained by the coupling of 5-HT2c receptors to $G\alpha_q$, which has been shown to stimulate neurotransmitter release (for review, see Berg et al., 1998). However, the 5-HT2c receptors have been shown to couple to other α subunits of the G-protein signalling complex (Alberts et al., 1999; Cussac et al., 2002). The 5-HT2c receptor can couple to the $G\alpha_i$ that elicits an inhibitory response in cells (Cussac et al., 2002). It still remains unclear which α subunit couples with the 5-HT2c receptor in

Fig. 7. Behavioural effects of Tat-3L4F and the 5-HT2cR agonist Ro600175. Data were presented as means \pm SEM ($n = 7$ for each group). (a, b) Measures of anxiety in the elevated plus maze. One-way ANOVA showed a significant overall difference in both entry ratio ($F_{3,24} = 4.769$, $p < 0.05$), the number of open arm entries per number of closed arm entries, and dwell ratio ($F_{3,24} = 3.168$, $p < 0.05$), the time in open arms per trial duration. Tukey *post hoc* test further revealed a significant increase of both entry ratio ($p < 0.05$) and dwell ratio ($p < 0.05$) in rats treated with Ro600175 (Ro, 3 mg/kg, i.p.) but not with either Tat-3L5F (3L5F, 0.1 μ mol/kg, i.p.) or Tat-3L4F (3L4F, 0.1 μ mol/kg, i.p.), relative to vehicle-injected rats, suggesting anxiogenic effects produced by Ro600175. (c) The food intake test was performed using a strategy of mild food deprivation for 3 hr near the beginning of the dark cycle, followed by quantification of the amount of food consumed in a 2-h period following drug treatment. While one-way ANOVA showed a significant overall difference ($F_{3,24} = 7.801$, $p < 0.05$), Tukey *post hoc* test revealed hypophagia effects ($p < 0.05$) produced by Ro600175 (Ro, 3 mg/kg, i.p.), but not by Tat-3L5F (3L5F, 0.1 μ mol/kg, i.p.) or Tat-3L4F (3L4F, 0.1 μ mol/kg, i.p.), relative to vehicle. (d) Locomotor activity levels were examined by computerized monitoring of photo-beam breaks in a square maze for 5 min after drug treatment. One-way ANOVA showed a significant overall difference ($F_{3,24} = 3.053$, $p < 0.05$), and Tukey *post hoc* test further revealed hypolocomotor activity ($p < 0.05$) produced by Ro600175 (Ro, 3 mg/kg, i.p.), but not by Tat-3L5F (3L5F, 0.1 μ mol/kg, i.p.) or Tat-3L4F (3L4F, 0.1 μ mol/kg, i.p.), relative to vehicle. (e) In inclined grid test, Ro600175 (Ro, 3 mg/kg, i.p.) significantly prolonged the latency for rats to move their both forepaws on an inclined grid frame (one-way ANOVA: $F_{3,24} = 4.200$, $p < 0.05$; Tukey *post hoc* test: $p < 0.05$), whereas Tat-3L5F (3L5F, 0.1 μ mol/kg, i.p.) or Tat-3L4F (3L4F, 0.1 μ mol/kg, i.p.) produced no significant difference relative to vehicle. (f) In the elevated beam-walking test, the time for rats to traverse the elevated wooden beam of 3-cm width and 1-m length was assessed following drug treatment. Relative to vehicle-treated rats, Ro600175-treated rats (Ro) showed a significant increase in time to traverse the beam (one-way ANOVA: $F_{3,24} = 6.200$, $p < 0.01$; Tukey *post hoc* test: $p < 0.01$), whereas rats treated with Tat-3L5F (3L5F) or Tat-3L4F (3L4F) exhibited no significant difference. * $p < 0.05$, as compared with any other group (Tukey *post hoc* test); ** $p < 0.05$, as compared with vehicle.

dopaminergic neurons in the VTA. It is possible that the 5-HT_{2c} receptor is coupled to G α_i in dopaminergic VTA neurons. Such a coupling would explain how the 5-HT_{2c} receptor expressed in dopaminergic neurons can result in decreased firing in the VTA.

An alternative model (reviewed in Müller and Carey, 2006) suggests that the coupling of PTEN and the 5-HT_{2c} receptor may indirectly regulate dopaminergic firing by regulating the activity of GABAergic neurons. In this particular model, a drug-induced decrease in the release of GABA in the VTA would be exacerbated by the dephosphorylation of the 5-HT_{2c} receptor by PTEN. In this particular model, activation from the 5-HT_{2c} receptor resulting from the uncoupling of PTEN would counteract the decrease of GABA release induced by drugs of abuse. The lines of evidence supporting this model stem from studies demonstrating that the 5-HT_{2c} receptor are expressed in GABAergic neurons. Studies by Eberle-Wang et al. (1997) demonstrate that 5-HT_{2c} mRNA does not co-localize with the mRNA for TH, but rather it co-localizes with mRNA for glutamic acid decarboxylase (GAD), a marker for GABA. These results were supported by immunohistochemistry results showing that the 5-HT_{2c} receptor protein co-localizes with GAD in the VTA (Bubar and Cunningham, 2007). This model, however, assumes that PTEN is expressed in GABAergic cells. Although results from our lab show that PTEN is expressed in dopaminergic neurons, it remains unclear if it is also expressed in VTA GABAergic neurons.

Abbreviations

5-HT	5-hydroxy-tryptamine
CPP	conditioned place preference
DA	dopamine
GAD	glutamic acid decarboxylase
GPCR	G-protein coupled receptor
IPSC	inhibitory postsynaptic currents
LTD	long-term depression
LTP	long-term potentiation
NAC	nucleus accumbens
NMDAR	N-methyl-D-aspartate receptor

PTEN	phosphate and tensin homologue deleted on chromosome 10
TH	tyrosine hydroxylase
THC	Δ^9 -tetrahydrocannabinol
VTA	ventral tegmental area

Acknowledgements

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and Natural Sciences and Engineering Research Council of Canada awarded to XZ, who is the recipient of the CIHR New Investigator Award and the Independent Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD) in the US.

References

- Alberts, G.L., Pregenzer, J.F., Im, W.B., Zaworski, P.G. and Gill, G.S. (1999) Agonist induced GTP γ 35S binding mediated by human 5-HT_{2c} receptors expressed in human embryonic kidney 293 cells. *Eur. J. Pharmacol.*, 383(3): 311–319.
- Alves, S.H., Pinheiro, G., Motta, V., landeira-Fernandez, J. and Cruz, A.P. (2004) Anxiogenic effects in the rat elevated plus-maze of 5-HT_{2c} agonists into ventral but no dorsal hippocampus. *Behav. Pharmacol.*, 15(1): 37–43.
- Backstrom, J.R., Price, R.D., Reasoner, D.T. and Sanders-Bush, E. (2000) Deletion of the serotonin 5-HT_{2c} receptor PDZ recognition motif prevents receptor phosphorylation and delays resensitization of receptor responses. *J. Biol. Chem.*, 275(31): 23620–23626.
- Berg, K.A., Maayani, S., Goldfarb, J. and Clarke, W.P. (1998) Pleiotropic behavior of 5-HT_{2A} and 5-HT_{2C} receptor agonists. *Ann. NY. Acad. Sci.*, 861: 104–110.
- Bubar, M.J. and Cunningham, K.A. (2007) Distribution of serotonin 5-HT_{2c} receptors in the ventral tegmental area. *Neuroscience*, 146(4): 286–297.
- Cameron, D.L. and Williams, J.T. (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. *J. Neurosci.*, 14(11): 6763–6767.
- Cheer, J.F., Marsden, C.A., Kendall, D.A. and Mason, R. (2000) Lack of response suppression follows repeated ventral tegmental cannabinoid administration: an in vitro electrophysiological study. *Neuroscience*, 99(4): 661–667.
- Clifton, P.G., Lee, M.D. and Dourish, C.T. (2000) Similarities in the action of Ro 60-0175, a 5-HT_{2C} receptor agonist, and D-fenfluramine on feeding patterns in the rat. *Psychopharmacology*, 152(3): 256–267.
- Cussac, D., Newman-Tancredi, A., Duqueyroux, D., Pasteau, V. and Millan, M.J. (2002) Differential activation of Gq/11

- and Gi3 proteins at 5-hydroxytryptamine_{2c} receptors revealed by antibody capture assays: influence of receptor reserve and relationship to agonist-directed trafficking. *Mol. Pharmacol.*, 62(3): 578–589.
- De Deurwaerdère, P., Navailles, S., Berg, K.A., Clarke, William P. and Spampinato, U. (2004) Constitutive activity of the serotonin_{2C} receptor inhibits in vivo dopamine release in the rat striatum and nucleus accumbens. *J. Neurosci.*, 24(13): 3235–3241.
- Di Giovanni, G., De Deurwaerdère, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin_{2c/2b} receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91(2): 587–597.
- Di Matteo, V., Cacchio, M., Di Giulio, C. and Esposito, E. (2002) Role of serotonin_{2C} receptors in the control of brain dopaminergic function. *Pharmacol. Biochem. Behav.*, 71(4): 727–734.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000) Biochemical and electrophysiological evidence that Ro 60-0175 inhibits mesolimbic dopaminergic function through serotonin_{2c} receptors. *Brain Res.*, 865(1): 85–90.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.F. (1997) Pattern expression of the serotonin 2C receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384(2): 233–247.
- Ferrari, F., Ottani, A. and Vivoli, R. (1999) Learning impairment produced in rats by the cannabinoid HU 210 in a water-maze task. *Pharmacol. Biochem. Behav.*, 64: 555–561.
- Fletcher, P.J., Chintoh, A.R., Sinyard, J. and Higgins, G.A. (2004) Injection of the 5-HT_{2c} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. *Neuropsychopharmacology*, 29(2): 308–318.
- French, E.D., Dillon, K. and Wu, X. (1997) Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport*, 8(3): 649–652.
- Freund, T.F., Kayona, I. and Piomelli, D. (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.*, 83(3): 1017–1066.
- Furnari, F.B., Lin, H., Huang, H.S. and Cavenne, W.K. (1997) Growth suppression of glioma cells by PTEN requires a functional phosphatase catalytic domain. *Proc. Natl. Acad. Sci. U.S.A.*, 94(23): 12479–12484.
- Gainetdinov, R.R., Premont, R.T., Bohn, L.M., Leftkowitz, R.J. and Caron, M.G. (2004) Desensitization of G protein-coupled receptors and neuronal functions. *Ann. Rev. Neurosci.*, 27: 107–144.
- Gardner, E.L. (2005) Endocannabinoid signaling system and brain reward: emphasis on dopamine. *Pharmacol. Biochem. Behav.*, 81: 263–284.
- Gervais, J. and Rouillard, C. (2000) Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse*, 35(4): 281–291.
- Gessa, G.L., Melis, M., Muntoni, A.L. and Diana, M. (1998) Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB₁ receptors. *Eur. J. Pharmacol.*, 341(1): 39–44.
- Grottick, A.J., Corrigan, W.A. and Higgins, G.A. (2001) Activation of 5-HT_{2c} receptors reduces the locomotor and rewarding effects of nicotine. *Psychopharmacology*, 157(3): 292–298.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295(3): 1183–1191.
- Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R. and Rice, K.C. (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiography study. *J. Neurosci.*, 11: 563–583.
- Higgins, G.A. and Fletcher, P.J. (2003) Serotonin and drug reward: focus on 5-HT_{2C} receptors. *Eur. J. Pharmacol.*, 480(1–3): 151–162.
- Hill, M.N., Froc, D.J., Fox, C.J., Gorzalka, B.B. and Christie, B.R. (2004) Prolonged cannabinoid treatment results in spatial working memory deficits and impaired long-term potentiation in the CA1 region of the hippocampus in vivo. *Eur. J. Neurosci.*, 20: 859–863.
- Hoffman, A.F. and Lupica, C.R. (2000) Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J. Neurosci.*, 20(7): 2470–2479.
- Hoffman, A.F. and Lupica, C.R. (2001) Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J. Neurophysiol.*, 85(1): 72–83.
- Hoffman, A.F., Riegel, A.C. and Lupica, C.R. (2003) Functional localization of cannabinoid receptors and endogenous cannabinoid production in distinct neuron populations of the hippocampus. *Eur. J. Neurosci.*, 18(3): 524–534.
- Ji, S.P., Zhang, Y., Van Cleemput, J., Jiang, W., Liao, M., Li, L., Wan, Q., Backstrom, J.R. and Zhang, X. (2006) Disruption of PTEN coupling with 5-HT_{2C} receptors suppresses behavioral responses induced by drugs of abuse. *Nat. Med.*, 12(3): 324–329.
- Johnson, S.W. and North, R.A. (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.*, 12(2): 483–488.
- Koob, G.F. and Le Moal, M. (1997) Drug abuse: hedonic homeostatic dysregulation. *Science*, 278(5335): 52–58.
- Lachyankar, M.B., Sultana, N., Schonhoff, C.M., Mitra, P., Poluha, W., Lambert, S., Queensberry, P.J., Litofsky, N.S., Recht, L.D., Nabi, R., Miller, S.J., Ohta, S., Neel, B.G. and Ross, A.H. (2000) A role for nuclear PTEN in neuronal differentiation. *J. Neurosci.*, 20(4): 1404–1413.
- Laviolette, S.R. and van der Kooy, D. (2004) The neurobiology of nicotine addiction: bridging the gap from molecule to behavior. *Nat. Rev. Neurosci.*, 5(1): 55–65.
- Lee, F.J., Xue, S., Pei, L., Vukusic, B., Chery, N., Wang, Y., Wang, Y.T., Niznik, H.B., Yu, X.M. and Liu, F. (2002) Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D₁ receptor. *Cell*, 111(2): 219–230.

- Lee, S.P., O'Dowd, B.F. and George, S.R. (2003) Homo- and hetero-oligomerization of G protein-coupled receptors. *Life Sci.*, 74(2–3): 173–180.
- Leslie, N.R. and Downes, C.P. (2004) PTEN function: how normal cells control it and tumour cells lose it. *Biochem. J.*, 382: 1–11.
- Liu, Q.S., Pu, L. and Poo, M.M. (2005) Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature*, 437(7061): 1027–1031.
- Maehama, T. and Dixon, J.E. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.*, 273(22): 13375–13378.
- Müller, C.P. and Carey, R.J. (2006) Intracellular 5-HT_{2c} receptor dephosphorylation: a new target for treating drug addiction. *Trends Pharmacol. Sci.*, 27(9): 455–458.
- Myers, M.P., Stolarov, J.P., Eng, C., Li, J., Wang, S.I., Wigler, M.H., Parsons, R. and Tonks, N.K. (1997) P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc. Natl. Acad. Sci. U.S.A.*, 94(17): 9052–9057.
- Nestler, E.J. (2004) Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. *Trends Pharmacol. Sci.*, 25(4): 210–218.
- Ning, K., Pei, L., Liao, M., Liu, B., Zhang, Y., Jiang, W., Mielke, J.G., Li, L., Chen, Y., El-Hayek, Y.H., Fehlings, M.G., Zhang, X., Liu, F., Eubanks, J. and Wan, Q. (2004) Dual neuroprotective signaling mediated by downregulating two distinct phosphatase activities of PTEN. *J. Neurosci.*, 24(16): 4052–4060.
- Pierucci, M., Di Matteo, V. and Esposito, E. (2004) Stimulation of serotonin 2C receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.*, 309(1): 109–118.
- Robbins, T.W. and Everitt, B.J. (1999) Drug addiction: bad habits add up. *Nature*, 398(6728): 567–570.
- Robbins, T.W. and Everitt, B.J. (2002) Limbic-striatal memory systems and drug addiction. *Neurobiol. Learn. Mem.*, 78(3): 625–636.
- Schlicker, E. and Kathmann, M. (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.*, 22(11): 565–572.
- Schwarze, S.R., Ho, A., Vocero-Akbani, A. and Dowdy, S.F. (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science*, 285: 1569–1572.
- Self, D.W., Choi, K.H., Simmons, D., Walker, J.R. and Smagula, C.S. (2004) Extinction training regulates neuroadaptive responses to withdrawal from chronic cocaine self-administration. *Learn. Mem.*, 11(5): 648–657.
- Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Sidrovski, D.P. and Mak, T.W. (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, 95(1): 29–39.
- Stanford, I.M., Kantaria, M.A., Chahal, H.S., Loucif, K.C. and Wilson, C.L. (2005) 5-hydroxytryptamine induced excitation and inhibition in the subthalamic nucleus: action at 5-HT_{2C}, 5-HT₄ and 5-HT_{1A} receptors. *Neuropharmacology*, 49(8): 1228–1234.
- Szabo, B., Siemas, S. and Wallmichrath, I. (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. *Eur. J. Neurosci.*, 15(12): 2057–2061.
- Wang, J.Q., Fibuch, E.E. and Mao, L. (2007) Regulation of mitogen-activated protein kinases by glutamate receptors. *J. Neurochem.*, 100(1): 1–11.
- Wang, Y., Cheng, A. and Mattson, M.P. (2006) The PTEN phosphatase is essential for long-term depression of hippocampus synapses. *Neuromolecular Med.*, 8(3): 329–336.
- Weng, L.P., Brown, J.L. and Eng, C. (2001) PTEN coordinates G(1) arrest by down-regulating cyclin D1 via its protein phosphatase activity and up-regulating p27 via its lipid phosphatase activity in a breast cancer model. *Hum. Mol. Genet.*, 10(6): 599–604.
- Westphal, R.S., Backstrom, J.R. and Sanders-Bush, E. (1995) Increase basal phosphorylation of the constitutively active serotonin 2C receptor accompanies agonist-mediated desensitization. *Mol. Pharmacol.*, 48(2): 200–205.
- Wong, T.P., Howland, J.G., Robillard, J.M., Ge, Y., Yu, W., Titterness, A.K., Brebner, K., Liu, L., Weinberg, J., Christie, B.R., Phillips, A.G. and Wang, Y.T. (2007) Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. *Proc. Natl. Acad. Sci. U.S.A.*, 104(27): 11471–11476.
- Wood, M.D. (2003) Therapeutic potential of 5-HT_{2C} receptor antagonists in the treatment of anxiety disorders. *Curr. Drug Targets CNS Neurol. Disord.*, 2: 383–387.
- Wu, X. and French, E.D. (2000) Effects of chronic delta9-tetrahydrocannabinol on rat midbrain dopamine neurons: an electrophysiological assessment. *Neuropharmacology*, 39(3): 391–398.
- Xu, L., Anwyl, R. and Rowan, M.J. (1997) Behavioural stress facilitates the induction of long-term depression in the hippocampus. *Nature*, 387(6632): 497–500.

CHAPTER 21

Serotonin modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders

Vincenzo Di Matteo¹, Massimo Pierucci¹, Ennio Esposito¹, Giuseppe Crescimanno²,
Arcangelo Benigno² and Giuseppe Di Giovanni^{2,*}

¹*Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud, 66030 Santa Maria, Imbaro (Chieti), Italy*

²*Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana G. Pagano, Università di Palermo, C.so Tuköry 129, 90134 Palermo, Italy*

Abstract: Several recent studies have emphasized a crucial role for the interactions between serotonergic and dopaminergic systems in movement control and the pathophysiology of basal ganglia. These observations are supported by anatomical evidence demonstrating large serotonergic innervation of all the basal ganglia nuclei. In fact, serotonergic terminals have been reported to make synaptic contacts with both substantia nigra dopamine-containing neurons and their terminal areas such as the striatum, the globus pallidus and the subthalamus. These brain areas contain a high concentration of serotonin (5-HT), with the substantia nigra pars reticulata receiving the greatest input. In this chapter, the distribution of different 5-HT receptor subtypes in the basal ganglia nuclei will be described. Furthermore, evidence demonstrating the serotonergic control of basal ganglia activity will be reviewed and the contribution of the different 5-HT receptor subtypes examined. The new avenues that the increasing knowledge of 5-HT in motor control has opened for exploring the pathophysiology and pharmacology of Parkinson's disease and other movement disorders will be discussed. It is clear that these avenues will be fruitful, despite the disappointing results so far obtained by clinical studies with selective 5-HT ligands. Nevertheless, these studies have led to a great increase in the attention given to the neurotransmitters of the basal ganglia and their connections.

Keywords: serotonergic receptors; basal ganglia; Parkinson's disease; motor disorders; dyskinesia; selective 5-HT drugs

Introduction

Since the 1950s, when serotonin (5-HT) was discovered in the mammalian central nervous

system (CNS), an enormous amount of experimental evidence has revealed the pivotal role of this biogenic amine in a bewildering diversity of behavioural and physiological processes. This is not surprising, considering the almost ubiquitous distribution of 5-HT-containing axon terminals throughout the CNS, although 5-HT is synthesized by a small group of neurons within the raphe

*Corresponding author. Tel.: (+39) 091655821;
Fax: (+39) 0616555823; E-mail: g.digiovanni@unipa.it

nuclei of the brain stem. Despite this broad axon-terminal domain of 5-HT neurons, a closer examination reveals a preferential targeting of motor areas in the CNS (Steinbusch, 1981). For example, in the rat, there is a very dense innervation of the ventral horn of the spinal cord, the motor nucleus of the trigeminal, the facial motor nucleus and all components of the basal ganglia circuitry (Lavoie and Parent, 1990). It is thus likely that 5-HT plays a role in regulating the appropriate selection of voluntary movements by the basal ganglia, and abnormalities in 5-HT transmission might contribute to the neural mechanisms underlying disorders of basal ganglia origin, such as Parkinson's disease (PD), Tourette's syndrome and obsessive compulsive disorder (Chapter 24; Ring and Serra-Mestre, 2002). Indeed, biochemical evidence suggests that 5-HT transmission is abnormal in the basal ganglia of patients with PD (Hornykiewicz, 1998) and, moreover, in the movement abnormalities generally associated with the use of L-3,4-dihydroxyphenylalanine [levodopa (L-DOPA)] and classical anti-psychotic drugs' (APDs) motor side effects (Bezard et al., 2001; Blackburn, 2004; Di Giovanni et al., 2006a; Chapters 22 and 23 in this volume).

During the last decades, advances in the understanding of receptors mediating the effect of 5-HT have represented one of the success stories of neuropharmacology. Many of the 5-HT receptors are found within the basal ganglia and most likely involved in the modulation of basal ganglia circuitry and in the pathology of their correlated disorders. Of particular interest with respect to the development of new treatments for PD and other motor disorders are the 5-HT_{1A/1B} and 5-HT_{2A/2C} receptor subtypes. This will be the subject of further discussion in the remainder of this chapter. First, the 5-HT innervations of the basal ganglia and the distribution of 5-HT receptors throughout the various nuclei will be summarized. Thereafter, several aspects of 5-HT control of the pathophysiology of basal ganglia nuclei will be discussed.

Therefore, it is clear that a detailed understanding of the neurotransmitter function in each condition is not merely academic but can lead to a

rationale for drug design and treatment strategies appropriate for that group of patients.

5-HT innervation of basal ganglia

More than 50 years have passed since Twarog and Page (1953) isolated an indole, identified as 5-HT, in the mammalian brain. Subsequently, Brodie et al. (1955) suggested that 5-HT might serve as a neurotransmitter in the CNS.

In vertebrates, the majority of the neurons containing 5-HT are grouped in nine nuclei named B1–B9, located in the medial part of the brain stem, generically called the raphe nuclei (Dahlström and Fuxe, 1964). These midline clusters can be divided into two major groups. The caudal or inferior group, localized in the medulla, contains the three nuclei projecting essentially to the grey matter of the spinal cord: the nucleus raphe magnus (NRM, cell group B5), nucleus raphe obscurus (NRO, cell groups B1, B2 and B3) and nucleus raphe pallidus (NRP, cell group B4). The rostral or superior group, located in the pons/mesencephalon, contains the dorsal raphe nucleus (DRN, cell groups B6 and B7) and the medial raphe nucleus (MRN, cell group B8). These nuclei supply about 80% of the serotonergic innervation to the forebrain. Even if, in many brain areas, the innervation coming from the two nuclei overlaps, in certain regions, the innervation comes exclusively or prevalently from one nucleus only. For example, the dorsal hippocampus receives a serotonergic innervation only from the MRN; other areas innervated preferentially from this nucleus are the medial preoptic area, the suprachiasmatic nucleus, the olfactory bulb and the medial septum nucleus. The DRN innervates all the basal ganglia circuitry (Fig. 1), sending projections to the corpus striatum, the globus pallidus (GP), the subthalamic nucleus (STN), the substantia nigra (SN) and the pedunculo-pontino nucleus (PPN), and provides most of the innervation of the prefrontal cortex, including the motor cortices. 5-HT-containing cell bodies of the raphe send projections to both dopaminergic (DAergic) and gamma-aminobutyric acidergic (GABAergic) cells in the SN and to their terminal

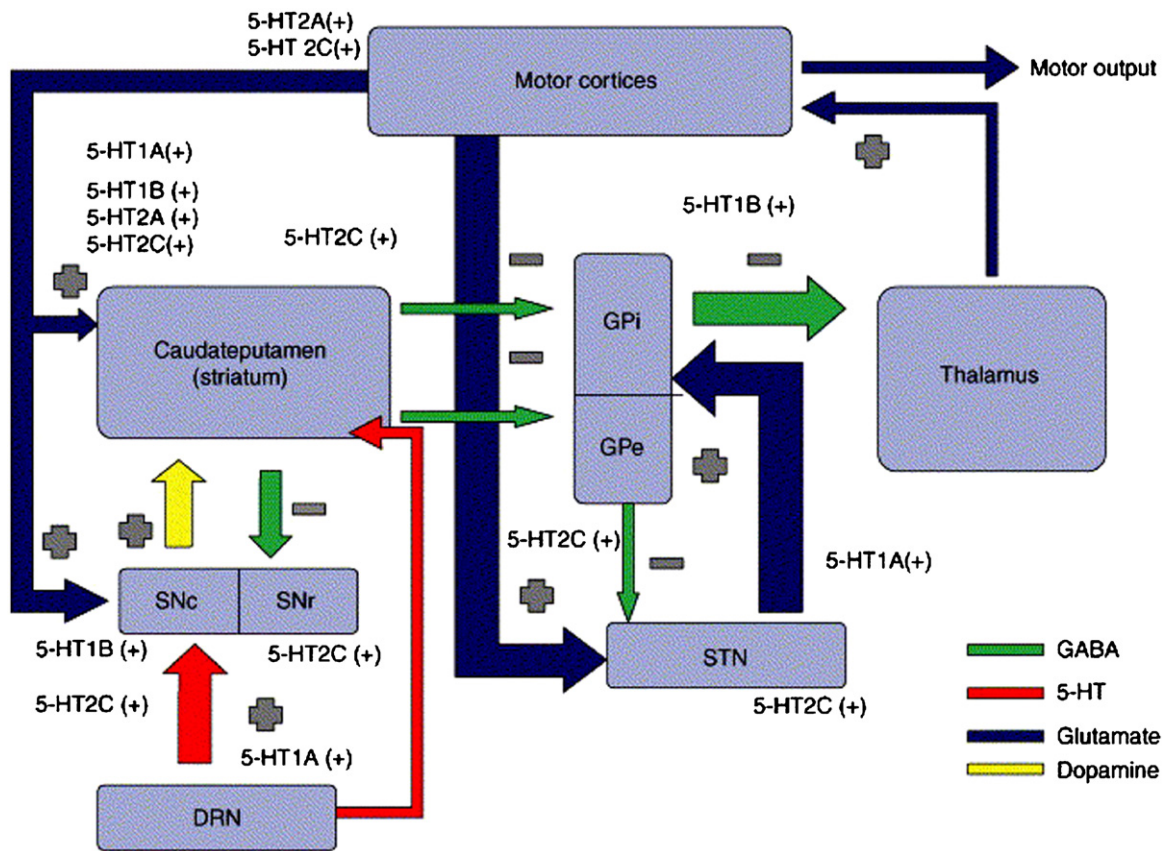


Fig. 1. Basal ganglia motor circuitry in Parkinson's disease (PD). 'Motor output' is used to generalize all descending motor pathways. During rest, the indirect motor output pathway (GPe→STN→GPi→thalamus) is primarily involved and the direct motor pathway (GPi→thalamus) is inactive. During periods of activity, the direct motor output pathway is primarily involved. The distribution of 5-HT receptor subtypes in the basal ganglia is indicated based on reports using a variety of methods. Reduced inhibition of the tonically active STN neurons results in increased inhibition of the thalamus via the pallidothalamic pathway. Intense GPi stimulation effectively inactivates this structure, reducing the inhibitory influence upon the thalamus by GPi. Yellow arrow indicates degenerating nigrostriatal pathway associated with PD; thin lines are pathways with reduced activity; medium-width lines are physiologically normal pathways; thicker lines are over-active pathways implicated in PD. Proposed sites of action of known 5-HT receptor subtype-selective compounds associated with PD and L-DOPA-induced dyskinesias are far from clear, but a more detailed understanding of our present knowledge is given in the main text for each 5-HT therapeutic strategy. SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; GPi, internal segment of the globus pallidus; GPe, external segment of the globus pallidus; DRN, dorsal raphe nucleus; STN, subthalamic nucleus; (+) denotes excitatory pathway; and (–) denotes inhibitory pathway. Adapted with permission from Blackburn (2004). (See Color Plate 21.1 in color plate section.)

fields (Hervé et al., 1987; Van Bockstaele et al., 1993; Moukhles et al., 1997). Moreover, electron microscopy demonstrates the presence of synaptic contacts of [³H]5-HT-labelled terminals with both DAergic and non-DAergic dendrites in the SN pars compacta (SNc) and reticulata (SNr) (Hervé et al., 1987; Moukhles et al.,

1997; Lee et al., 2000). The DRN innervates, together with the MRN, the ventral part of the hippocampus, the nucleus accumbens and various nuclei of the thalamus, among them the ventral lateral nuclear group that processes motor information and the hippocampus (Azmitia and Segal, 1978; Jacobs and Azmitia,

1992; McQuade and Sharp, 1995). Moreover, extensive serotonergic connections between the DRN and the MRN also exist (Jacobs and Azmitia, 1992).

5-HT receptors' distribution within the basal ganglia nuclei

A vast amount of research has led to the discovery and characterization of a plethora of 5-HT receptor subtypes. At present, seven classes of 5-HT (5-HT₁₋₇) receptors have been identified, which comprise at least 15 subtypes (Hoyer et al., 2002) (Table 1). This is not surprising, since with so many potential targets distributed throughout the CNS, 5-HT is a major neurotransmitter involved in such a large number of physiological and pathological processes.

The distribution of 5-HT receptors among the various basal ganglia structures has been widely investigated. Autoradiographic, in situ hybridization and binding studies, as well as

functional and pharmacological investigations, showed a differential distribution of the various 5-HT receptor subtypes both within the basal ganglia nuclei and among the different mammalian species that were used in these studies (Waeber et al., 1990a). Strikingly, the described pattern of distribution and expression of these receptors is modified in the animal model of pathologies involving basal ganglia circuitry as well as in human patients. Nevertheless, this could be no more than an epiphenomenal effect.

Striatum

High concentrations of both receptor mRNA and protein of different 5-HT receptor subtypes have been found in the striata of various species.

5-HT_{1A} was one of the first 5-HT receptor subtypes that was identified and pharmacologically characterized, and its distribution among different species of mammals has been widely investigated. So far, most of the receptor autoradiography, radioimmunohistochemistry and in

Table 1. Serotonin receptor subtypes

Receptor	Type of receptor	Effector mechanisms	Subtypes	Location in the basal ganglia	Speculated function
5-HT ₁	G protein linked	Inhibits adenylyl cyclase, opens K ⁺ channels	5-HT _{1A}	Caudate-putamen, subthalamic nucleus	Anxiety, depression
			5-HT _{1B}	Caudate-putamen, substantia nigra, globus pallidus	Locomotion
			5-HT _{1D}	Substantia nigra	Locomotion
			5-HT _{1E}	Caudate-putamen	
			5-HT _{1F}	Caudate-putamen	
5-HT ₂	G protein linked	Stimulation of phospholipase C, closing of K ⁺ channels	5-HT _{2A}	Nucleus accumbens, caudate-putamen	
			5-HT _{2B}	None detected in the basal ganglia	
			5-HT _{2C}	Nucleus accumbens, caudate-putamen, substantia nigra, subthalamus	
5-HT ₃	Ligand-gated cation channel	Na ⁺ current	5-HT _{3A} ,	GABAergic projection neurons of caudate-putamen	Anxiety, depression, emesis
			5-HT _{3B} ,		
			5-HT _{3C}		
5-HT ₄	G protein linked	Stimulation of adenylyl cyclase	5-HT ₄	GABAergic projection neurons of caudate-putamen	Anxiety, depression
5-HT ₅	G protein linked	Inhibits adenylyl cyclase	5-HT _{5A} ,	Caudate-putamen	Motor control, feeding, anxiety, depression, learning, memory
			5-HT _{5B}		
5-HT ₆	G protein linked	Stimulation of adenylyl cyclase	5-HT ₆	Dendrites of GABAergic striatopallidal and striatonigral neurons	Dopamine transmission
5-HT ₇	G protein linked	Stimulation of adenylyl cyclase	5-HT ₇	Caudate-putamen, nucleus accumbens	Locomotion, circadian rhythms

situ hybridization studies have reported low or barely detectable levels of labelling for both 5-HT_{1A} receptor protein and mRNA in the caudate-putamen of the rat (Miquel et al., 1991; Riad et al., 1991; Kung et al., 1995; Wright et al., 1995), mouse (Schiller et al., 2003) and primate brain (Mengod et al., 1996). The same regional distribution has been found in the caudate and putamen nuclei of human brain, using postmortem radiolabelling and in vivo positron emission tomography (PET) with various radiotracers (Pike et al., 1995; Pasqualetti et al., 1996; Hall et al., 1997; Duncan et al., 1998; Ito et al., 1999). However, a recent study reported a patchy distribution of 5-HT_{1A} receptors confined to striosomes of the primate striatum characterized by poor calbindin immunostaining. In these compartments, the receptor density was equal to that in other enriched brain areas, such as some hippocampal fields, and it was increased in a 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-lesioned primate model of PD, suggesting a key role for this receptor subtype in the control of movement by the basal ganglia (Frechilla et al., 2001).

The 5-HT_{1B} receptor subtype localization in the striatum has been widely demonstrated in various mammal brains. In rats and mice, early [³H]5-HT binding studies, using selective ligands as displacing agents for the 5-HT_{1A} receptor subtype, showed intermediate-to-low concentrations of 5-HT_{1B} binding or immunoreactivity sites in the caudate-putamen (Pazos and Palacios, 1985; Blurton and Wood, 1986; Verge et al., 1986; Waeber et al., 1989; Middlemiss and Hutson, 1990; Sari et al., 1999). In a first attempt to characterize the distribution of 5-HT receptors in the brains of different mammal species, autoradiographic data seemed to indicate a lack, in cats, guinea pigs, monkeys and humans, of receptors with a pharmacological profile similar to that of the rat and mouse 5-HT_{1B} (Hoyer et al., 1986; Pazos et al., 1987a; Waeber et al., 1989). Similarities in the 5-HT_{1D} distribution in the brains of these species to that of 5-HT_{1B} in rats and mice led to the hypothesis that these two receptor subtypes were species homologues (Waeber et al., 1989; del Arco et al., 1993). Subsequent findings, relative to the

molecular cloning of 5-HT₁ receptor subtypes, showed the presence, in human brain, of two genes expressing different receptors displaying the pharmacology of the originally described 5-HT_{1D} sites, named 5-HT_{1D α} and 5-HT_{1D β} (77% sequence homology in the transmembrane domain). 5-HT_{1D β} showed a 96% homology to the cloned rodent 5-HT_{1B} receptor. Following this new experimental evidence, the 5-HT_{1B/1D} receptor nomenclature underwent a reassessment (for reviews, see Barnes and Sharp, 1999; Hoyer et al., 2002). According to the new classification, comparative studies showed binding sites for this receptor, as well as high hybridization signals for its mRNA, in the caudate-putamen of rodent brain (Bruinvels et al., 1994; Bonaventure et al., 1998) and the corresponding caudate and putamen in humans (Varnas et al., 2001, 2005); moreover, some authors showed a dorsoventral gradient in the distribution of both this receptor and its mRNA, with higher levels in the ventral as compared to the dorsal striatum (Compan et al., 1998; Varnas et al., 2001, 2005) in both rats and humans. According to the authors, this evidence, together with the complete lack of mRNA in both the SN and ventral pallidum, seems to indicate a localization of the 5-HT_{1B} receptor subtype on axon terminals arising from medium spiny neurons (MSNs) of the ventral striatal regions, thus confirming previous immunoreactivity and in situ hybridization studies in mouse and rat brain (Boschert et al., 1994; Sari et al., 1999). On the other hand, binding sites in the striatum may reflect both somatodendritic and presynaptic localizations of this receptor on striatal neurons (projection neurons and/or interneurons) or on thalamic/cortical afferents, respectively (Bonaventure et al., 1998).

The presence of 5-HT_{1D} (namely, 5-HT_{1D α}) receptor and mRNA in the striatum of rodents, primates and humans has been reported, even if weaker levels of both protein-binding and hybridization signals were observed with respect to the 5-HT_{1B} (Bruinvels et al., 1993a). Similarly to 5-HT_{1B} receptors, some authors described a discrepancy in the distribution pattern of 5-HT_{1D} mRNA and its protein in the striatum, SN and GP of mouse brain and therefore hypothesized a

presynaptic localization of 5-HT_{1D} receptors in the latter two areas (Boschert et al., 1994). Finally, regarding the 5-HT₁ receptor family, Bruinvels et al. (1993b) reported the presence of the 5-HT_{1E} receptor subtype mRNA in both the caudate nucleus and putamen of primates, showing stronger hybridization signals in monkey brain than that obtained in human brain.

Both 5-HT_{2A} receptor subtype and its mRNA have been extensively demonstrated to be present in the striatum of various mammal species. Moreover, the described distribution pattern showed increasing gradients for this receptor in the rostrocaudal and mediolateral directions (Pazos et al., 1985, 1987b; Mengod et al., 1990; Pompeiano et al., 1994; Wright et al., 1995; Lopez-Gimenez et al., 1997). In human brain, using the high-affinity radioligand [³H]MDL 100907, a high density of labelling has been shown to be distributed in patches throughout the caudate nucleus and putamen (Lopez-Gimenez et al., 1999). Regarding cellular distribution, the 5-HT_{2A} receptors showed a somatodendritic localization, as demonstrated also by the correlation between the distribution of 5-HT_{2A} protein and its mRNA (Cornea-Hébert et al., 1999). Furthermore, a more prominent localization in dendrites than in cell bodies was found in the dorsolateral caudate-putamen of rat brain, using immunocytochemistry (Rodríguez et al., 1999).

A number of studies have demonstrated a widespread distribution of the 5-HT_{2C} receptor subtype in rat, monkey and human brains, particularly among the different basal ganglia structures. Moreover, marked differences have been revealed in the distribution of 5-HT_{2C} mRNA and its level of expression within the different subregions of the basal ganglia (Hoffman and Mezey, 1989; Mengod et al., 1990; Wright et al., 1995; Eberle-Wang et al., 1997). Most labelled neurons in the striatum were efferent medium-sized neurons but not cholinergic interneurons, although recent polymerase chain reaction (PCR) evidence instead revealed high expression of 5-HT_{2C}, 5-HT₆ and 5-HT₇ mRNAs in cholinergic interneurons (Bonsi et al., 2007a).

Neurons expressing 5-HT_{2C} mRNA have been found in discrete areas of the caudate-putamen

showing no preferential localization with substance P, dynorphin or enkephalin, thus indicating that 5-HT_{2C} receptors are not differentially expressed on the two major striatal output pathways (striatonigral and striatopallidal projection neurons). On the other hand, with regard to patch/matrix striatal structures, 5-HT_{2C} mRNA showed a preferential localization in the patch compartment areas, suggesting a role for this receptor in the modulation of striatal projections to the SNc (Gerfen, 1984, 1985; Ward and Dorsa, 1996).

Using various radioligands, some authors have reported the presence of 5-HT₃ receptors in the striatum of different mammals, albeit higher receptor densities have been found in the human caudate nucleus and putamen than in the corresponding structures of the rat brain (Barnes et al., 1990; Gehlert et al., 1991; Laporte et al., 1992; Abi-Dargham et al., 1993; Bufton et al., 1993; Parker et al., 1996; Morales et al., 1998; Fletcher and Barnes, 1999; Marazziti et al., 2001). Binding studies on homogenates of human putamen suggested a localization of these receptors on neurons that have their cell bodies within this region, such as GABAergic projection neurons, but not on DAergic neurons. Indeed, patients with diagnosed Huntington's disease showed decreased binding densities in the striatum, unlike those affected by DAergic cell loss associated with PD (Steward et al., 1993). Moreover, as for other brain areas, data obtained using synaptosomes isolated from rat striatum showed the presence in this area of functional presynaptic 5-HT₃ receptors, besides the known postsynaptic localization (Nichols and Mollard, 1996; Ronde and Nichols, 1998; Nayak et al., 1999).

An analysis of the distribution of the 5-HT₄ receptor showed its presence in several components of the basal ganglia of different mammal species. Generally, the distribution pattern of the 5-HT₄ receptor binding sites in the striatum matched that observed for its mRNA in all the different species used in binding and in situ hybridization studies. However, some species' differences have been shown. Thus, ventromedial-to-dorsolateral-increasing gradients of labelling densities have been observed in the rat and mouse brain caudate-putamen (Waeber et al.,

1994; Vilaro et al., 1996), while it was less pronounced in the same structure of guinea pig brain; similarly, monkey and human caudate nuclei and putamens showed very high densities of receptor and mRNA binding sites but no apparent gradient in their distribution (Bonaventure et al., 2000; Vilaro et al., 2005). The decrease in binding densities following selective lesion of rat caudate-putamen indicated a somatodendritic localization of 5-HT₄ receptors on GABAergic projection neurons and cholinergic/GABAergic interneurons in this structure; on the other hand, following 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway, there were no changes in striatal levels of receptor binding, clearly indicating that this receptor was not localized on DAergic terminals (Compan et al., 1996; Vilaro et al., 2005). These data are in agreement with postmortem studies on brains of patients with Huntington's disease or PD, showing a decrease in receptor binding only in the first group of patients (Reynolds et al., 1995; Vilaro et al., 2005).

Only moderate-to-low expression of 5-HT_{5A} receptor protein and mRNA has been found in the striatum of mouse, rat and human brain (Boess and Martin, 1994; Rees et al., 1994; Wesolowska, 2002). In particular, 5-HT_{5A} immunoreactivity was detectable at low levels in MSNs of rat caudate-putamen (Oliver et al., 2000).

On the other hand, the 5-HT₆ receptor subtype has been shown to be particularly abundant in this area of the basal ganglia. High levels of both protein and mRNA expression have been found in the caudate-putamen of rat and pig brain (Monsma et al., 1993; Ruat et al., 1993a; Ward et al., 1995; Kohen et al., 1996; Yoshioka et al., 1998; Hirst et al., 2000) as well as in the caudate nucleus and putamen of human brain (Kohen et al., 1996; Hirst et al., 2003), thus demonstrating a similar distribution pattern for this receptor in the brains of these species. In contrast, only low levels of both 5-HT₆ receptor protein and mRNA have been found in the same brain areas of two different strains of mice (Hirst et al., 2003). As the regional distribution of 5-HT₆ receptor generally matched that found for the 5-HT₆ receptor mRNA, it is likely that the former is mainly

localized on somas and/or dendrites of neurons. This conclusion is further supported by light and electron microscopy immunoreactivity data clearly showing the localization of this receptor on dendritic processes of spiny neurons in the striatum (Gerard et al., 1997; Hamon et al., 1999). Finally, selective lesion of 5-HT neurons by injection of 5,7-dihydroxytryptamine (5,7-DHT) in the DRN has been shown to not affect the levels of 5-HT₆ mRNA in the striatum of rat brain, thus confirming the postsynaptic localization of this receptor with respect to 5-HT neurons innervating this area (Gerard et al., 1996). Similarly, the lack of any difference in the levels of protein binding, following 6-OHDA selective lesion of the nigrostriatal pathway, demonstrates that the 5-HT₆ is also not located on membranes of DA neurons (Roberts et al., 2002).

Finally, the presence of the 5-HT₇ receptor subtype has been reported in the striatum of rodents as well as in the caudate nucleus and putamen of human brain, with a protein distribution pattern generally matching that of its mRNA. In addition, some species' differences in receptor densities were reported between human and rodent brain (Ruat et al., 1993b; Wesolowska, 2002; Martin-Cora and Pazos, 2004; Varnas et al., 2004).

Substantia nigra and globus pallidus

Very low levels of binding have been found in the SN or the GP of mouse, rat and human brains for both 5-HT_{1A} receptor protein and its mRNA (Khawaja, 1995; Kung et al., 1995; Wright et al., 1995; Hall et al., 1997; Schiller et al., 2003). Interestingly, some authors showed high binding of [³H]zolpidem in the rat SN in contrast to low binding observed in the same region of human brain (Duncan et al., 1998). By contrast, among the basal ganglia nuclei, the highest levels of 5-HT_{1B} protein bindings were found in both the SN and the GP of different mammal species. Unlike the striatum, in situ hybridization studies failed to reveal the presence of 5-HT_{1B} mRNA in these structures. Taken together, therefore, these data seem to suggest a presynaptic localization of

this receptor subtype, presumably on terminals of striatal afferents (Pazos et al., 1987a; Boschert et al., 1994; Bruinvels et al., 1994; Bonaventure et al., 1998; Sari et al., 1999; Varnas et al., 2001, 2005), where it acts as heteroreceptor (Barnes and Sharp, 1999). Moreover, the levels of 5-HT_{1B} binding sites in these areas after selective lesion of nigral DAergic cells or striatal neurons, as well as radioligand studies on postmortem human brain of patients who had suffered degenerative movement disorders, clearly demonstrate the localization of this receptor on striatonigral and striatopallidal GABAergic afferents (Waeber et al., 1990b; Castro et al., 1998; Compan et al., 1998). Similarly to the 5-HT_{1B} subtype, radiolabelled sites for 5-HT_{1D} receptors were found in these structures, despite the lower densities as compared to the previous receptor (partially due to the lack of specific radioligand), while *in situ* hybridization studies showed a lack of its mRNA (Bruinvels et al., 1993a, 1994). Again, these data, together with receptor and mRNA distribution in the striatum previously reported, are in line with a putative localization on axon terminals in these areas.

Regarding the 5-HT₂ family, intermediate levels of 5-HT_{2A} receptor mRNA were found in the SNc, SNr and pars lateralis of rat brain (Mengod et al., 1990; Pompeiano et al., 1994; Wright et al., 1995). Regarding receptor distribution, as mentioned above, there is a general concordance between the distributions of 5-HT_{2A} protein and its mRNA. Early studies found high concentrations of [³H]ketanserin binding in the SNc and the SNr in rats while only very low concentrations of this receptor have been shown in the GP (Pazos et al., 1985, 1987b). Nevertheless, some recent immunohistochemistry and autoradiography studies found lower levels of 5-HT_{2A} receptors expressed in these areas (Cornea-Hébert et al., 1999; Lopez-Gimenez et al., 1999; Bubser et al., 2001). These discrepancies in receptor concentrations in the SN could be explained by the use in previous studies of some radioligands, such as [³H]ketanserin, which has been shown to have a high affinity for non-serotonergic sites, as well as its known selectivity for the 5-HT₂ receptor family (Lopez-Gimenez et al., 1997). Finally, the presence of this receptor

subtype in the GP of rat brain has been confirmed by recent immunohistochemistry data showing that about 70% of neurons expressing the 5-HT_{2A} receptor project to the striatum (Bubser et al., 2001). Neither 5-HT_{2C} mRNA nor receptor protein has been found in the GP or in the entopeduncular nucleus (EPN) while neurons showing labelling for this receptor have been found in both the SNc and the SNr. Furthermore, the distribution pattern of 5-HT_{2C} in this area showed a marked rostrocaudal labelling gradient, characterized by higher receptor densities in caudal regions of both nuclear subdivisions. Finally, with regard to cellular localization, receptor expression seems to be confined to GABAergic neurons but not to DAergic cell bodies (Van Bockstaele et al., 1994; Van Bockstaele and Pickel, 1995; Steffensen et al., 1998; Di Giovanni et al., 2001).

The 5-HT₃ receptor subtype has been detected by membrane binding essays and immunolabelling in the SN of both rat and human brain (Laporte et al., 1992; Bufton et al., 1993; Gehlert et al., 1993; Doucet et al., 1999). Nevertheless, only lightly detectable receptor densities have been shown for this area.

The presence of 5-HT₄ receptor protein, but not its mRNA, has been reported in both the GP and the SN of different mammal species, with the highest densities in the latter area, thus supporting the idea of a localization of this receptor on striatopallidal and striatonigral terminals (Compan et al., 1996; Ullmer et al., 1996; Vilario et al., 1996; Bonaventure et al., 2000). Moreover, 5-HT₄ receptor binding sites in the rat SN were confined to the pars lateralis, while in guinea pig brain, a wider distribution was observed in the same area, with most of the SNr being strongly labelled (Waeber et al., 1994; Vilario et al., 2005); in human and monkey brain, the distribution pattern resembled that of guinea pig brain.

Finally, with regards to the last three 5-HT receptor families, only moderate-to-low concentrations of both receptor proteins and mRNA have been found in the SN and the GP of rat and human brain for the 5-HT_{5A} (Rees et al., 1994; Oliver et al., 2000), 5-HT₆ (Kohen et al., 1996;

Gerard et al., 1997; Hamon et al., 1999; Roberts et al., 2002; Hirst et al., 2003) and 5-HT₇ (Ruat et al., 1993b; Martin-Cora and Pazos, 2004; Varnas et al., 2004) receptor subtypes.

Subthalamic nucleus

So far, little evidence exists regarding the presence of moderate-to-low concentrations of 5-HT_{1A}, 5-HT₄, 5-HT₅ and 5-HT₆ receptors and/or their mRNA in the STN (Pompeiano et al., 1992). By contrast, high hybridization signals were found for both 5-HT_{1B} and 5-HT_{2C} receptor mRNAs in the rodent brain (Bruinvels et al., 1994; Eberle-Wang et al., 1997).

5-HT modulation of basal ganglia circuitry

The 5-HT modulation of DAergic nigrostriatal function, in terms of control of DA SNc neuron firing discharge and DA release in the striatum, has been extensively reviewed in Chapters 2 and 3. Therefore, we will not discuss this subject and we refer the readers to these two chapters.

5-HT modulation of striatal activity

In agreement with anatomical data, most of the reports on this subject indicate that striatal cells are predominantly affected by dorsal raphe (DR) stimulation, with the median raphe stimulation being unable to alter striatal cell activity directly (Olpe and Koella, 1977; Davies and Tongroach, 1978). In addition, it has been found that suppression of spontaneous firing activity is the main response of cultured striatal cells (Yakel et al., 1988) in vivo following DRN stimulation, and excitation being observed in only about 10% of them. Nevertheless, a rebound excitation was observed in some cells that were initially inhibited. Local application of 5-HT was found to produce changes similar to that caused by raphe stimulation, and most of the cells responsive to the raphe stimulation were also affected by nigral stimulation (Davies and Tongroach, 1978). However, using intracellular recording techniques, Kitai and co-workers (Vandermaelen et al., 1979) found that

DRN stimulation was consistently capable of generating excitatory postsynaptic potentials. These findings were subsequently repeated in the laboratory by the authors, and it was indicated that non-5-HT DRN-striatal neurons could be involved in striatal responses to 5-HT. They also showed that the increase of the firing frequency in rat neostriatal MSNs induced by 5-HT depended on reducing voltage-dependent potassium currents (Park et al., 1982; Stefani et al., 1990; Wilms et al., 2001).

Stimulation of postsynaptic 5-HT_{1A} receptors by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OHDPAT) induced an increase in locomotor activity (Mignon and Wolf, 2002). Moreover, Gerber et al. (1988) and Matsubara et al. (2006) reported that 5-HT_{1A} receptor stimulation has an anti-parkinsonian effect in 6-OHDA-lesioned rats, inducing a robust contralateral rotational behaviour. Current theories of circling behaviour hypothesize that the animal turns away from the basal ganglia output, where activity has been reduced. Moreover, the stimulation of 5-HT_{1A} receptors has been shown to be capable of inducing contralateral rotation also following degeneration of the DRN (Blackburn et al., 1984; Gerber et al., 1988). This rotational behaviour has been explained by a supersensitivity of 5-HT_{1A} receptors in the SNc of the DA-lesioned rats, resulting in an increase of DA in the striatum (Blackburn et al., 1984; Gerber et al., 1988). In agreement with this theory, tandospirone, a highly potent and selective 5-HT_{1A} receptor agonist, remarkably potentiated the contralateral turning induced by apomorphine (Matsubara et al., 2006). Nevertheless, it has been shown that 8-OHDPAT can also induce ipsilateral turning in unilateral 6-OHDA-lesioned rats (Mignon and Wolf, 2002, 2007).

The discrepancies notwithstanding these findings are valuable in demonstrating the potential utility of drugs that possess 5-HT_{1A} in the symptomatic treatment of PD. The 5-HT_{1A} agonists might inhibit 5-HT release, acting pre-synaptically (Gerber et al., 1988), or have a postsynaptic action (Lucas et al., 1997; Matsubara et al., 2006), decreasing striatal glutamate release from corticofugal projections, without involving

direct activation of D₂ receptors (Antonelli et al., 2005; Mignon and Wolf, 2005, 2007). Similarly, WAY 100135 increased glutamate (GLU) outflow in the striatum of rats (Dijk et al., 1995). These findings suggest that 5-HT_{1A} receptor stimulation could give rise to a decrease in the corticofugal glutamate drive (e.g. to the striatum or the STN) that could ultimately be manifested as decreased activation of the output nuclei of the basal ganglia (medial GPi and SNr). A reduction in excitatory drive to these output nuclei would lead to a disinhibition of the motor thalamus, thereby ameliorating the motor deficits of PD (Mignon and Wolf, 2007). These results suggest that 5-HT_{1A} agonists could have therapeutic potentials for the treatment of PD by modulating neuronal activities of non-DAergic pathways, such as the excitatory amino acid pathways in the basal ganglia.

5-HT released from serotonergic terminals in the striatum exerts a negative feedback on its own neuronal activity via 5-HT_{1B} as well as 5-HT_{1A} autoreceptors. Indeed, stimulation of 5-HT_{1B} receptors, localized on the terminals of 5-HT neurons, reduced 5-HT release and also modulated L-DOPA metabolism to DA in the striatum (Knobelman et al., 2000; Carta et al., 2007). Several *in vivo* electrophysiological and neurochemical studies suggest an important role of the 5-HT_{1B} receptor in modulating the activity of mesostriatal DAergic neurons. Indeed, 5-HT_{1B} receptor stimulation enhances striatal DAergic activity, generally attributed to an inhibition of GABA release and a consequent disinhibition of DA neuronal activity (see Chapters 2 and 3). Rats with unilateral DRN lesions showed contralateral turning in response to the putative 5HT_{1B} agonist RU 24969, while a much weaker effect was revealed in 6-OHDA-lesioned rats (Gerber et al., 1988). 5-HT_{1A} and 5-HT_{1B} receptors appear to act synergistically in reducing 5-HT transmission in the basal ganglia, and it is important to note that low doses of agonists for these receptors are able to suppress dyskinesia, without affecting the anti-parkinsonian effect of L-DOPA in the presence of spared DA terminals, suggesting an early use of these drugs to counteract the development of dyskinesia in PD patients (see Chapter 22).

The 5-HT₂ family has been intensively investigated in the striatum. Compelling data by Blier and colleagues (el Mansari et al., 1994; el Mansari and Blier, 1997) concerning single-unit recordings coupled with microiontophoresis *in vivo*, in rats, guinea pigs and 5-HT_{2C} receptor mutant mice, support the hypothesis of an inhibitory action of the 5-HT system on the neuronal activity of the striatum [presumably MSNs] that through the activation of 5-HT₂ receptors. These authors investigated only in the head of the caudate nucleus and reported an inhibitory effect of 5-HT, mimicked by the 5-HT_{2A} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the 5-HT_{2C} receptor agonist m-chlorophenylpiperazine (mCPP) (el Mansari et al., 1994; el Mansari and Blier, 1997). It is noteworthy that significantly less quisqualate was required to activate neurons in the caudate nucleus of 5-HT₂ mutant mice than in the wild-type mice, suggesting that 5-HT_{2C} receptors serve a tonic inhibitory role in membrane excitability (Rueter et al., 2000). Strikingly, neither the selective 5-HT_{2A} agonist MDL 100907 nor clozapine antagonized DOI or mCPP in the caudate nucleus in mice. The authors suggested that DOI and mCPP might be acting in the caudate nucleus through an atypical 5-HT₂ receptor yet to be characterized and a hypothesis that has not been investigated by successive studies. More likely, this lack of antagonism depends on the pharmacological design of the experiments. Indeed, in rats, el Mansari and Blier (1997) showed that the inhibitory effect of DOI, but not that of mCPP, was antagonized by a 4-day treatment with metergoline and ritanserin, indicating that the suppressant effect of DOI may be mediated by 5-HT_{2A} receptors in the head of the caudate nucleus. Contrary evidence has been shown by an *in vivo* study in which only excitation of the striatal neurons induced by microiontophoretic application of 5-HT was revealed, while DOI caused a preferential inhibitory response, highlighting diverse effects of 5-HT in different parts of the striatum (Wilms et al., 2001).

Recently, the role of serotonergic control on striatal cholinergic interneurons has been explored *in vitro* (Blomeley and Bracci, 2005; Bonsi et al.,

2007a). Striatal acetylcholine (ACh), and its interplay with DA, has long been recognized as playing a crucial role in voluntary movement (Duvoisin, 1967). In the striatum, ACh is mainly released by a population of large aspiny interneurons (LAIs) (Phelps et al., 1985; Zhou et al., 2002; Tepper and Bolam, 2004). Pakhotin and Bracci (2007) have shown that LAIs exerted a strong inhibitory control over the neighbouring striatal MSNs via inhibition of their GLUergic input. These effects are presynaptic and mediated by both M_2 and M_3 muscarinic receptors, both present on corticostriatal terminals. 5-HT strongly and reversibly increased spontaneous firing rates of LAIs in vitro via a reversible reduction of two pharmacologically and kinetically distinct after-hyperpolarizations (AHPs) that play an important role in limiting the excitability of cholinergic interneurons (Blomeley and Bracci, 2005). Blomeley and Bracci (2005), furthermore, showed that 5-HT₂ receptors were responsible for the excitatory effects of 5-HT in cholinergic interneurons, although they could not identify the 5-HT receptor subtype involved since they used α -methyl-5-HT and ketanserin, highly unspecific ligands. Recently, Bonsi et al. (2007a) managed to rule out the role of 5-HT_{2A} showing that only the pretreatment with the selective 5-HT_{2C} antagonist RS 102221 caused a significant reduction in the 5-HT-induced depolarization of cholinergic interneurons. This evidence was strongly supported by PCR analysis data showing that the 5-HT_{2C} receptors are expressed by about 80% of LAIs in contrast to a sporadic expression of the 5-HT_{2A} subtypes. Accordingly, the response of striatal cholinergic interneurons to the 5-HT was partially blocked by phospholipase C (PLC) inhibitor, the transduction pathways linked to the 5-HT_{2C} receptor subtypes. Thus, 5-HT induces cell depolarization and increase in firing frequency, acting through postsynaptic 5-HT_{2C} receptors, probably causing inhibition of K^+ currents or increase of a cationic conductance in striatal cholinergic interneurons. In the light of these recent results, 5-HT_{2C} agonists might reduce striatal MSN activity indirectly via an increase of the inhibitory cholinergic tone. Moreover, 5-HT₆ and 5-HT₇ receptor subtypes are also involved in the potent

excitatory effect of 5-HT but not in that of 5-HT₃ and 5-HT₄ (Bonsi et al., 2007a). Therefore, modulating the activity of cholinergic striatal interneurons by 5-HT_{2C}, 5-HT₆ or 5-HT₇ receptors may have positive therapeutic benefits for motor diseases.

In concordance with these inhibitory 5-HT effects on MSNs through the activation of 5-HT_{2C} receptors, there is the behavioural evidence that caudate injections of 5-HT provoked contraversive turning, while conversely, intracaudate methysergide induced ipsiversive circling (James and Starr, 1980). In addition, the facilitation by hyoscine and the attenuation by eserine of the 5-HT-induced contraversive circling, together with the converse effects of these drugs on methysergide-evoked ipsiversive rotations, are consistent with raphe-caudate 5-HT fibres synapsing directly with, and exciting, striatal cholinergic neurons. Given that motor behaviour recruits multiple striatal neurotransmitter systems, recent attention has focused on the interaction between DA and 5-HT receptors. In the intact striatum, several studies have consistently demonstrated that intrinsic 5-HT₂ receptors can modify DA function and have postulated divergent roles for 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Lucas et al., 2000; Porrás et al., 2002). 5-HT_{2A} antagonists reduce hyperlocomotion induced by cocaine, amphetamine and 3,4-methylenedioxymethamphetamine (MDMA) (Kehne et al., 1996; O'Neill et al., 1999), whereas 5-HT_{2C} receptor antagonists have been shown to enhance or reduce these effects, depending upon the compounds and neuronal sites studied (Filip and Cunningham, 2002; Fletcher et al., 2002; Filip et al., 2004). Recent studies by Walker's group have shown that, following DA depletion, D₁-induced locomotor activity can be reduced by antagonism of striatal 5-HT_{2A}, but not 5-HT_{2C}, receptors (Bishop et al., 2005). This preferential involvement of the 5-HT_{2A} subtype is confirmed by the evidence that intrastriatal injections of the selective 5-HT_{2C} antagonist RS 102221 had no effect on motor activity; conversely, the 5-HT_{2A} agonist DOI induced motor behaviour in neonatal 6-OHDA-lesioned rats, which was attenuated by the 5-HT_{2A} receptor antagonism (Bishop et al., 2004).

Interestingly, recent pharmacological data and lesion studies have established that the biosynthesis of neuropeptides in the striatum is regulated by the 5-HT innervation originating from the DRN, suggesting that 5-HT in the striatum might exert a metabolic regulatory function on the biosynthesis of neuropeptides rather than acting as an ion channel modulator (Horner et al., 2005; D'Addario et al., 2007).

5-HT modulation of SNr activity

The SNr neurons receive the largest 5-HT innervation from the DRN of all brain regions (Fibiger and Miller, 1977; Corvaja et al., 1993) and express a high level of 5-HT receptors, which are both postsynaptic and located on the somatodendritic region of SNr neurons and postsynaptic on terminals of SNr inputs. It has been calculated that the density of 5-HT-immunoreactive varicosities in the SNr is in the order of $9 \times 10^6/\text{mm}^3$, of which about 74% form synaptic specializations with GABAergic projection neurons (Moukhles et al., 1997). This picture gives an idea of the great influence that 5-HT could have on the activity of SNr neurons. Early studies have revealed a primarily inhibitory action of 5-HT on SNr neurons. Electrical stimulation of the DRN caused mostly inhibitory responses in the SNr, as measured by single-unit extracellular recordings in vivo (Fibiger and Miller, 1977). Similarly, the iontophoresis of 5-HT into the SN produced mixed, although mostly inhibitory, effects in the SNr (Dray et al., 1976; Collingridge and Davies, 1981). The inhibition of SNr neurons by 5-HT is supported by the finding that unilateral injection of 5-HT, 5-HT_{1D} agonist and selective serotonin reuptake inhibitors (SSRIs) into the SNr of freely moving rats elicited a contraversive circling behaviour (James and Starr, 1980; Blackburn et al., 1981; Oberlander et al., 1981; Higgins et al., 1991; Bata-Garcia et al., 2002) as muscimol does (Oberlander et al., 1981). In spite of this evidence, Lacey and co-workers have shown, by using in vitro electrophysiological methods, that 5-HT not only directly excites SNr neurons but also disinhibits them by reducing GABA release from striatonigral terminals, acting on presynaptic

5-HT_{1B} receptors (Rick et al., 1995; Stanford and Lacey, 1996). On the other hand, a subsequent electrophysiological in vitro study demonstrated also a direct inhibitory action of 5-HT on SNr neurons (Gongora-Alfaro et al., 1997). 5-HT and the 5-HT-uptake inhibitor duloxetine reduced the firing rate of the majority of SNr neurons recorded, suggesting that synaptically released endogenous 5-HT act directly on 5-HT_{1B} receptors located in these neurons. The agonists that mimicked that effect were only of the 5-HT_{1B} class (CP 93129 and TFMPP). Neither the 5-HT₂ antagonist ritanserin nor the GABA_A antagonist bicuculline were able to block that inhibition, suggesting that, in addition to an indirect action (Stanford and Lacey, 1996), some SNr neurons may be directly inhibited by 5-HT.

The 5-HT-induced excitation observed in the majority of the SNr neurons recorded is most probably mediated by a direct action on 5-HT₂ receptors being blocked by ketanserin and ritanserin and mimicked by α -methyl-5-HT, unselective antagonists and agonists of the 5-HT₂ receptor subtype (Rick et al., 1995; Stanford and Lacey, 1996). In addition, Gongora-Alfaro and colleagues' in vitro study revealed that 5-HT could excite about half of the SNr neurons tested; this effect was seen in the neurons blocked by methysergide, thus confirming the involvement of 5-HT₂ receptors (Gongora-Alfaro et al., 1997). The above experimental evidence, although underlining a pivotal role for the 5-HT₂ receptor subtype in the modulation of SNr neurons, does not discriminate the involvement of different subtypes. We have tried to answer this question, and consistent with the aforementioned evidence, we showed that selective 5-HT_{2C} activation excites SNr neurons in vivo (Di Giovanni et al., 2001; Invernizzi et al., 2007). This effect was evident after both systemic administration and local microiontophoretic application of mCPP and Ro 60-0175 (Di Giovanni et al., 2001; Invernizzi et al., 2007). As further confirmation of a selective activation of 5-HT_{2C} receptors, excitatory effects of mCPP and Ro 60-0175 were blocked by pretreatment with SB 242084 and SB 243213, potent and selective 5-HT_{2C} antagonists. An interesting finding of our first study was the differential effect exerted by

mCPP on subpopulations of SNr neurons. Thus, mCPP caused a marked excitation of the so-called P(0) non-DA neurons in the SNr, whereas it did not affect the P(+) neurons. These neurons are identified on the basis of the presence P(+) or the absence P(0) of an excitatory response to a noxious stimulus (footpinch). There is evidence that P(+) neurons in the SNr are GABAergic interneurons that exert a direct inhibitory influence on DA neurons in the SNc, whereas P(0) cells represent SNr projection neurons. Thus, mCPP caused a marked excitation of presumed SNr projection neurons but did not modify the SNr interneuron firing discharge (Di Giovanni et al., 2001). These data have been confirmed using Ro 60-0175, the most selective agonist to date, which caused excitation only in half of the SNr neurons recorded, although no information about the territory of their innervations was investigated (Invernizzi et al., 2007). Nevertheless, it is most likely that the SNr neurons excited by the 5-HT_{2C} agonist Ro 60-0175 are the P(0)-projecting neurons that responded to mCPP treatment (Di Giovanni et al., 2001). Consistent with these electrophysiological data, both systemic and intranigral administration of Ro 60-0175 and mCPP markedly increased extracellular GABA levels in the SNr, while glutamate levels were not affected. The stimulatory effect of systemic and local Ro 60-0175 on GABA release was dependent on the ongoing neuronal activity [tetrodotoxin (TTX) sensitive] and completely prevented by systemic administration of SB 243213. On the other hand, local application of SB 243213 into the SNr only partially blocked Ro 60-0175-induced GABA release. This suggests that the control exerted by 5-HT_{2C} receptors on extracellular GABA in the SNr involves both intra- and extranigral components, such as the striatonigral pathway (Invernizzi et al., 2007). Based on our *in vivo* (Di Giovanni et al., 2001; Invernizzi et al., 2007) and *in vitro* (Rick et al., 1995; Stanford and Lacey, 1996; Gongora-Alfaro et al., 1997) evidence, it is possible to speculate that 5-HT released *in vivo* elicits a direct excitatory response in a discrete population of SNr neurons, probably resulting in the expression of 5-HT_{2C}, which in turn inhibits a greater number of neighbouring SNr cells through

GABA release from their extensive axon collaterals (Mailly et al., 2003; Invernizzi et al., 2007). Therefore, the source of GABA in the SNr might have many different origins — i.e. it might derive from a subpopulation of GABA-containing neurons in the SNr that are excited by 5-HT_{2C} agonists, from release of GABA by the somatodendritic regions and their recurrent collaterals and/or from the GPe neurons excited, in turn, by the STN. We can exclude a striatal GABA source since its neurons are principally inhibited by 5-HT_{2C} receptor agonists.

The overall effect of activation of 5-HT_{2C} receptors, therefore, would be the overinhibition of nigrothalamic GABAergic neurons and consequent decrease of GABA levels in the motor thalamus. According to the current model of basal ganglia functional organization (DeLong, 1990), reduction of the subthalamonigral GLUergic excitatory drive and/or increase in the GABAergic inhibitory influence on nigrothalamic GABAergic neurons lead to disinhibition of thalamocortical GLUergic projections and movement initiation (Deniau and Chevalier, 1985). Thus, drugs acting at 5-HT receptors might be useful in treating akinesia and other parkinsonian symptoms characterized by an overactivity of the nigrothalamic pathway (Deniau and Chevalier, 1985; DeLong, 1990).

Strikingly, under physiological conditions, 5-HT_{2C} receptors do not exert any tonic control on the basal ganglia activity, although blocking these receptors in the striatum leads to an increase of DA release. Indeed, there is evidence that a number of selective 5-HT_{2C} antagonists, such as SB 200646A, SB 206553 and SB 242084, do not elicit locomotory activity, when given alone (Kennett et al., 1994, 1996, 1997). Moreover, intranigral infusion of the antagonist SB 206553 into the SNr on the unlesioned side of a 6-OHDA-lesioned rat did not elicit a significant rotational response (Fox et al., 1998).

On the other hand, it has been shown that 5-HT_{2C} receptor transmission may be a key determinant in the activity of SNr in parkinsonian basal ganglia. Accordingly, infusion of SB 206553 into the SNr on the 6-OHDA-lesioned side elicited a marked rotational response contraversive to

the injection (Fox et al., 1998). Such behaviour represents a reduction in the activity of basal ganglia outputs and can be taken as representing a potential anti-parkinsonian action. Moreover, systemic administration of SB 206553 enhanced the action of D₂ agonist quinpirole and D₁ agonist SKF 82958 in eliciting a rotational response contraversive to the lesioned side (Fox et al., 1998; Fox and Brotchie, 2000a). The mechanism whereby 5-HT_{2C} receptor antagonists enhance the anti-parkinsonian action of DA receptor agonists may involve reducing the overactivity of the SNr. When given alone, 5-HT_{2C} receptor antagonists may be capable of reducing the activity only to a certain degree following systemic administration. Therefore, there may not be a sufficient reduction in the activity of the SNr to restore the normal thalamocortical output and have an overt anti-parkinsonian effect.

From these findings, it is clear that in the 6-OHDA PD model, the antagonists at 5-HT_{2C} receptors show anti-parkinsonian effects and are probably decreasing SNr activity, an effect obtained in normal rats with the agonists instead. We explained this paradox suggesting that under pathological conditions, when the basal ganglia circuitry is impaired by DA depletion, the 5-HT_{2C} receptor transmission is also altered. The over-expression of 5-HT_{2C} receptors in the SNr could lead to a clear-cut excitatory effect on the output structures since the indirect collateral inhibition is totally overcome. Thus, the consequence is a contribution to the SNr overactivity that is known to be a hallmark of PD and related disorders (Di Giovanni et al., 2006b). 5-HT_{2C} receptor transmission may be a key determinant in the activity of the SNr in parkinsonian basal ganglia and its selective activation in this condition might have a surprising opposite effect compared to that which it has on the 'normal' circuitry. Studies are underway in our laboratories to verify this supposition.

In addition, the blockade of 5-HT_{2A/2C} receptors is a determinant of the effect of clozapine and risperidone in inhibiting the discharge of SNr neurons (Bruggeman et al., 2000). Indeed, Bruggeman et al. (2000) showed that concurrent 5-HT_{2A/2C} and moderate DA D₂ receptor anta-

gonism can mimic the in vivo effects of these atypical antipsychotics on the firing rate of SNr neurons. Therefore, the inhibitory effect of the atypical antipsychotics clozapine and risperidone and of concurrent 5-HT₂/D₂ antagonism on the SNr may reflect a mechanism to counteract motor side effects [extrapyramidal symptoms (EPS)] by disinhibiting thalamocortical circuits.

On the other hand, it could also be a mechanism to alleviate negative symptoms. This is based on the fact that the SNr, aside from prominent innervations from the dorsolateral striatum, also receives afferents from the nucleus accumbens, innervating subfields of the mediodorsal and ventromedial thalamic nuclei mainly affiliated to the prelimbic area and the prefrontal cortex. Therefore, one must consider that changes in SNr activity may reflect not only motor activity — and in that sense EPS — but also emotional and motivational processes, which may be involved in negative symptoms.

5-HT modulation of STN activity

The STN is an important mediator of the output circuits subserving basal ganglia motor function, and a potent link between the serotonergic system, the STN and motor behaviour has been highlighted. It is interposed in the direct pathway between the external segment of the GP (GPe) and the GP (GPi)/SNr. The STN also has projections that interact with the other primary output pathway from the striatum, the indirect pathway, at the level of the GPe (Kita and Kitai, 1987; Shink et al., 1996). This connectivity provides the STN with a unique ability to mediate basal ganglia motor function, and accordingly, the STN has a strong influence on motor behaviour related to basal ganglia DAergic neurotransmission. For instance, the STN has been implicated in the mediation of parkinsonian movement disorders. An increase in the basal activity of the excitatory GLUergic afferent neurons of the STN, associated with the loss of DA terminals in the striatum, may play a role in the hypokinetic symptoms of this condition (Smith and Grace, 1992). Indeed, STN lesion, as well as its inactivation by deep brain stimulation (DBS), has shown to have anti-parkinsonian

effects in an experimental primate model of PD and in PD patients (Krack et al., 1998; Charles et al., 2004; Sturman et al., 2004). Under normal DA function, in both humans and primates, unilateral lesions of the STN result in hemiballism and chorea, which are characterized by involuntary, hyperkinetic movements of the contralateral limbs (Mitchell et al., 1985; Bhidayasiri and Truong, 2004). During the past decade, patients suffering from PD have undergone modulation of this STN hyperactivity by high-frequency stimulation (HFS) (Limousin et al., 1995). HFS of the STN has dramatic therapeutic effects on locomotor symptoms (Krack et al., 2003). The mechanism by which STN HFS improves locomotor symptoms is not well understood, but some evidence suggests that HFS modulates the pathological activity within the STN (Garcia et al., 2005), lowering GABA release in the motor thalamus (Stefani et al., 2006).

On the other hand, it is known that 5-HT neurons, mainly from the DRN, innervate the STN and clearly modulate its neuronal activity. 5-HT may have multiple actions in the STN. Whole-cell patch-clamp and extracellular single-unit recordings on rat brain slices with selective 5-HT agonists and antagonists indicated both 5-HT_{1A} receptor-mediated inhibitory and 5-HT_{2C} and 5-HT₄ receptor-mediated excitatory responses of 5-HT in subthalamic neurons (Stanford et al., 2005; Shen et al., 2007). In addition, 5-HT inhibits synaptic transmission in the STN by activating presynaptic 5-HT_{1B} receptors. Indeed, in a recent electrophysiological study in slices of rat brain, 5-HT reduced the amplitude of both GLUergic excitatory postsynaptic currents (EPSCs) and GABAergic inhibitory postsynaptic currents (IPSCs) on the STN neuron membrane (Shen and Johnson, 2008). The 5-HT-induced inhibition of synaptic currents was associated with a significant increase in the paired-pulse ratios of evoked EPSCs and IPSCs, suggesting that 5-HT acts presynaptically to suppress both GLU and GABA release. However, 5-HT was more potent for reducing EPSCs compared to IPSCs (Shen and Johnson, 2008). This inhibitory effect was mediated via the activation of 5-HT_{1B} receptors because selective 5-HT_{1B} antagonists blocked

5-HT-induced inhibition of EPSCs and IPSCs (Shen and Johnson, 2008). Consistent with its presynaptic location, 5-HT_{1B} receptor activation has been shown to cause presynaptic inhibition of GABA-mediated transmission in the SN also (Johnson et al., 1992; Stanford and Lacey, 1996). According to a widely used model of basal ganglia function, a reduction in excitatory glutamate input to the STN would be expected to improve the symptoms of PD (Bonsi et al., 2007b). Indeed, injection of 5-HT_{1B} agonists systemically (Oberlander et al., 1987; Rempel et al., 1993) or into the STN (Martinez-Price and Geyer, 2002) has been reported to increase locomotion in rats. However, 5-HT_{1B} receptor stimulation has also been reported to reduce L-DOPA-induced dyskinesia (LID) in a rat model of PD (Carta et al., 2007), which is not what one would predict based on inhibition of excitatory input to the STN. Moreover, 5-HT_{1B} agonists have also been reported to interfere with the benefit of L-DOPA in a marmoset model of PD. Activation of 5-HT_{1B/1D} receptors induced motor deficits and inhibited motor responses to L-DOPA, whereas blockade of 5-HT_{1B} receptors had no observable effects on motor behaviours (Jackson et al., 2004). These data suggest that neither stimulation nor blockade of 5-HT_{1B} receptors will be therapeutically beneficial to the treatment of PD or drug-induced dyskinetic syndromes.

The 5-HT_{2C} receptors are most likely to be involved in 5-HT effects since they are present in a relatively high concentration in this nucleus. The most frequent response to 5-HT seems to be an excitation of STN neurons (Flores et al., 1995; Stanford et al., 2005; Xiang et al., 2005; Shen et al., 2007). This effect is mediated by the activation of the 5-HT_{2C} and 5-HT₄ receptors being reversed by the combined use of selective antagonists for 5-HT₄ and 5-HT_{2C} receptors (Stanford et al., 2005; Xiang et al., 2005). In addition, Shen et al. (2007) found that STN neuron burst firing was facilitated by 5HT_{2C}- and 5HT₄-dependent currents, and since excessive burst firing of STN neurons has been implicated in the expression of the symptoms of PD, it was suggested that antagonists at 5HT_{2C} or 5HT₄ receptors might be useful in the treatment of PD. Moreover, an

inhibitory action of 5-HT over a small subpopulation (about 20%) of STN neurons has been also shown (Stanford et al., 2005; Shen et al., 2007), and it seems to be mediated by 5-HT_{1A} receptor activation (Stanford et al., 2005). Thus, this electrophysiological evidence indicates that 5-HT-induced excitation and inhibition in the STN are separate entities and most likely to arise as a consequence of independent, direct postsynaptic effects mediated by 5-HT_{2C}, 5-HT₄ and 5-HT_{1A} subtypes. The excitatory 5-HT effect through the activation of 5-HT_{2C} receptors is in accordance with the results of a recent study that investigated the effect of subthalamic DBS, using clinically relevant stimulation parameters, on DOI-induced hypomobility (Hameleers et al., 2007). These authors found that administration of DOI decreased the locomotor activity, as evidenced by a net decrease in the distance moved, the velocity and the time spent in moving. This decrease in locomotion was reversed by DBS of the STN. Varying results of DOI administration on locomotion have been obtained in the past. Some studies showed an increase in locomotor activity after DOI administration (Darmani et al., 1996; Granoff and Ashby, 1998); some authors found no effects (Hawkins et al., 2002), and others demonstrated hypomobility in rats treated with DOI (Krebs-Thomson and Geyer, 1996). It is probably the concentration of DOI that is contributing to the difference in these studies. In their study, Xiang et al. (2005) found a clear reduction of locomotor activity, which was reversed by STN HFS. It is known that injection of 5-HT_{2A} and 5-HT_{2C} agonists can increase the firing rate of STN neurons (Xiang et al., 2005). Since DOI activates these receptors, it is likely that DOI excites STN neurons and induces increased STN activity. Increased STN activity is thought to be responsible for the hypokinesia in PD. In addition, these findings support the hypothesis that 5-HT₂ receptors may mediate the therapeutic effects of STN HFS on locomotor symptoms.

However, this evidence is not in accordance with a large body of behavioural evidence that shows an inhibitory action of 5-HT over the STN by acting on 5-HT_{2C} receptors. As a result, a decrease in the excitatory input from the STN to GPe/SNr occurs,

which in turn enhances the activity of the ipsilateral motor thalamus. Indeed, the unilateral injection of 5-HT into the STN induces a contralateral dose-dependent turning behaviour, which is blocked by the unselective 5-HT₂ antagonist mianserin. The contribution of the 5-HT_{2C} receptor in 5-HT-induced behaviour was revealed by the intrasubthalamic injection of the 5-HT_{2C} receptor agonist MK 212 that, in concordance, increased the net turns (Belforte and Pazo, 2004). In addition, the blockade of subthalamic 5-HT_{2C} receptors suppressed the stereotypic behaviour induced by apomorphine administration (Barwick et al., 2000) while both systemic administration and local unilateral infusion of mCPP into the STN induced an increase in oral movements in rats (Eberle-Wang et al., 1996; De Deurwaerdere and Chesselet, 2000; Mehta et al., 2001) that resemble the orofacial dyskinesias occurring as a severe side effect of prolonged treatment with antipsychotic drugs in humans (Waddington et al., 1986; Ellison, 1991). Oral dyskinesia observed after peripheral injections of mCPP was enhanced by 5,7-DHT-induced lesion of the serotonergic neurons, probably due to an altered sensitivity to 5-HT_{2C} receptor stimulation in the STN (Mehta et al., 2001). Interestingly, these authors observed mCPP-induced seizure-like behaviours in a subset of lesioned rats that were never observed in sham-lesioned animals, thus demonstrating a pivotal role for the 5-HT_{2C} receptor in the control of the normal neuronal excitability, a phenomenon already noted by others (Mehta et al., 2001). Despite the fact that the mechanism by which serotonergic inputs to the STN contribute to its normal functioning remains controversial, the behavioural data discussed above clearly suggest that excess stimulation of 5-HT_{2C} receptors in this region may lead to hyperkinetic movement disorders. Thus, 5-HT_{2C} antagonists can be useful to treat the side effects of long-term administration of neuroleptics in schizophrenia (Tarsy and Baldessarini, 1984; Reynolds, 2004). Serotonergic projections from the DRN and the MRN innervate all components of the basal ganglia circuitry; thus, there is evidence that endogenous 5-HT induces excitations of the STN neurons through several types of 5-HT receptors (Belforte and

Pazo, 2004; Stanford et al., 2005; Xiang et al., 2005). Since this nucleus is considered to be a major driving force in the basal ganglia circuit (Albin et al., 1989; Utter and Basso, 2008), it is important to understand the role of the various 5-HT receptor subtypes in the control of this area. With regard to the other 5-HT receptors, besides 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C}, there is evidence that only the 5-HT₃ and 5-HT₄ receptors are involved in the control of the subthalamic neurons. Unilateral injection of 5-HT into the STN induced a contralateral dose-dependent turning behaviour, attributed to a decreased excitatory input from the STN to the SNr, which in turn enhanced the activity of the ipsilateral motor thalamus (Belforte and Pazo, 2004). Similar results were also observed with microinjections of quipazine, a mixed 5-HT_{2B/2C/3} agonist, MK 212, a 5-HT_{2B/2C} agonist, and m-chlorophenylbiguanidine, a 5-HT₃ agonist (Belforte and Pazo, 2004). Furthermore, kainic acid lesion of the SNr suppressed the contralateral rotations elicited by the stimulation of 5-HT_{2B/2C} and 5-HT₃ subthalamic receptors (Belforte and Pazo, 2004). Taken together, these data suggest that 5-HT tonically stimulates the subthalamonigral pathway, through 5-HT_{2C} and 5-HT₃ receptor subtypes, without involving the DAergic innervation of the nucleus, because stimulation of subthalamic 5-HT receptors in animals bearing a lesion of the nigrostriatal pathway did not modify this motor response (Belforte and Pazo, 2004).

The stimulating action of 5-HT on the STN is further confirmed by electrophysiological investigations. Endogenous application of 5-HT in mouse and rat brain slices increased the firing frequency of subthalamic neurons (Flores et al., 1995; Stanford et al., 2005; Xiang et al., 2005).

The increased firing rate of these neurons was attributed to the depolarization of membrane potential caused by a reduction of potassium conductance mediated by 5-HT_{2C} and mainly by 5-HT₄ receptor subtypes (Stanford et al., 2005; Xiang et al., 2005). Using several serotonergic agonists and antagonists, Xiang et al. (2005) found that only α -methyl-5-HT, a 5-HT₂ agonist with good affinity for 5-HT₄ receptors, and cisapride, a 5-HT₄ agonist, mimicked the action of 5-HT.

Furthermore, the 5-HT action was partially reversed by the 5-HT₄ antagonist SB 23597-190, the 5-HT₂ antagonist ketanserin and the 5-HT_{2C} receptor antagonist RS 102221; in addition, the effect of RS 102221 was comparable with that of ketanserin. Therefore, it was concluded that 5-HT₄ and 5-HT_{2C} receptor subtypes are involved in the excitatory action of 5-HT in STN neurons and may be co-localized in a single neuron (Xiang et al., 2005). These data were replicated by Stanford et al. (2005), who showed that the pre-infusion of RS 102221 and GR 113808, another 5-HT₄ antagonist, reduced excitations of STN neurons induced by local application of 5-HT. On the basis of these results, it was concluded that when there is the cortical activation for a specific movement, a group of raphe serotonergic neurons that project to the STN and STN neurons themselves might be simultaneously activated, then amplify subthalamic activity and consequently facilitate the excitation of the output structures in the basal ganglia, which in turn inhibits the thalamic motor area (Xiang et al., 2005). In the classical rate model of basal ganglia function, the neural mechanisms underlying the generation of parkinsonian symptoms are thought to involve reduced activation of primary motor and premotor cortex, and supplementary motor areas secondary to an over-activation of the output regions of the basal ganglia, i.e. SNr and GPi (Albin et al., 1989), largely because of excessive excitatory drive from the STN, consequent to DA loss in the striatum (Nicholson and Brotchie, 2002; Utter and Basso, 2008). Hence, it is theoretically possible that antagonists at the 5-HT_{2C} and 5-HT₄ receptors, which act directly to reduce STN neural activity, may have positive therapeutic benefits in PD.

5-HT modulation of GP activity

Anatomical and experimental evidence supports a pivotal role for 5-HT in the control of neuronal activity of both the GPe and the GPi, which express several 5-HT receptors. Perkins and Stone (1983) found that typical high-frequency firing GPe neurons in anaesthetized rats were not responsive to iontophoretically applied 5-HT, and

5-HT inhibited a small number of low-frequency firing GPe neurons. In contrast, Querejeta et al. (2005) found that local application of a 5-HT receptor agonist, or fluoxetine, excites most of the GPe neurons in anaesthetized rats, showing the presence of a serotonergic excitatory tone on GP neurons. This evidence was further confirmed by a recent patch-clamp recording study (Chen et al., 2008). In reality, the effect of 5-HT is more complex because of the existence of various receptor subtypes on presynaptic and postsynaptic membranes in the pallidum. In fact, recent electrophysiological evidence has shown that 5-HT exerts strong modulation on inhibitory and excitatory responses to cortical stimulation in the GPe and the GPi (Kita et al., 2007; Hashimoto and Kita, 2008). 5-HT suppressed GABAergic inhibitory responses to cortical stimulation in monkeys, through presynaptic 5-HT_{1B} receptors, densely expressed on axons and axon terminals in the pallidum (Kita et al., 2007). On the other hand, 5-HT_{1A} receptors are involved in the suppression of GLUergic excitations of the GP because local application of WAY 100635, a 5-HT_{1A} selective antagonist, blocked the effect of subsequent applications of 5-carboxamindotryptamine (5-CT) in suppressing cortical stimulation-induced excitations (Kita et al., 2007). Hence, 5-HT may reduce ionotropic GLUergic excitation, probably through 5-HT_{1A} receptors located at presynaptic and/or postsynaptic sites. On pallidal neuron slices, 5-HT and 5-CT presynaptically reduce glutamate release in the GPe, and the antagonistic effect of GR 55562 suggested an involvement of 5-HT_{1B/1D} receptors in this effect (Hashimoto and Kita, 2008).

In accordance with these findings, an in vivo electrophysiological study reported that local application of the 5-HT_{1B} receptor agonist L-694247 excited most GPe neurons, which can be due to presynaptic suppression of GABAergic inhibitions, in anaesthetized rats (Querejeta et al., 2005). In addition, the effect of 5-HT in the electrical activity of GP neurons of rats with unilateral quinolinic acid striatal lesions was severely attenuated, indicating that presynaptic 5-HT_{1B} receptors modulate GABA release from striatopallidal terminals (Querejeta et al., 2005). Taken together, these data indicate that the tonic

activation of 5-HT_{1B} receptors significantly contributes to the decrease of GABA release from striatopallidal GABAergic terminals facilitating GP neurons' spiking; on the other hand, the blockade of these receptors causes a significant decrease on the spiking frequency of GP neurons due to the augmented GABA release from striatopallidal terminals (Querejeta et al., 2005). In line with these studies, 5HT_{1B} receptor activation with CP-93129 inhibited the release of [³H]-GABA from pallidal slices, and intrapallidal injection of CP-93129 alleviated akinesia in the reserpine-treated rat model of PD, indicating that some 5HT_{1B} receptors can function as heteroreceptors in the GP, reducing the release of GABA from striatopallidal neurons. This cellular mechanism underlies the anti-akinetic activity of CP-93129 seen in the reserpine-treated rat model of PD (Chadha et al., 2000).

The application of 5-HT into the superfusion medium of brain slices containing GP neurons directly stimulated the receptors of the recorded neurons and produced a reversible depolarization of their membrane that consequently increased the firing rate of these neurons (Chen et al., 2008). 5-HT postsynaptic excitation of pallidal neurons occurs through activation of 5-HT₄ or 5-HT₇ receptors but not via 5-HT_{2C} and 5-HT₃ receptors (Bengtson et al., 2004; Kita et al., 2007; Chen et al., 2008; Hashimoto and Kita, 2008).

These findings support the hypothesis that the increase of 5-HT tone, which mainly excites pallidal neurons directly and counteracts the inhibition from the striatum selectively, will exert an anti-parkinsonian effect.

5-HT in PD and other motor disorders

Parkinson's disease

PD is the second most common neurodegenerative disease in the elderly population with an inevitable exitus. The idiopathic form is a progressive disorder, the impact of which reaches far beyond the clinical signs and symptoms exhibited by those afflicted. Clinical features at presentation include the asymmetric onset of cardinal motor symptoms

such as tremor at rest, bradykinesia, muscular rigidity, stooped posture and instability (Sian et al., 1999).

Since Hornykiewicz's pioneering work in identifying the SNc as the site of major pathological change in PD, reduced DAergic innervation of the striatum has been thought to be central to its pathogenesis (Hornykiewicz, 1973). Hitherto, the underlying mechanisms of neuronal loss in patients are not known. Therefore, current therapies work mainly to alleviate symptoms rather than to halt the progression of the disease (Di Giovanni, 2008). There have been major advances in understanding the aetiopathogenesis of PD and the modalities whereby the neurodegenerative process begins and progresses; therefore, the development of drugs to slow and halt DAergic neuronal degeneration or even to prevent the disease now seems realistic (Esposito et al., 2007a, b, c; Di Giovanni, 2008).

The modulation of 5-HT of the basal ganglia nuclei has obvious implications for the treatment of a range of motor diseases, most notably including PD, LID and antipsychotic-induced extrapyramidal effects. It has become axiomatic that manipulation of 5-HT transmission may be pivotal in treating the symptoms and of key importance in improving symptomatology in this patient set (Nicholson and Brotchie, 2002; Di Giovanni et al., 2006a, b; Scholtissen, et al., 2006).

Although 5-HT involvement in PD has long since been known (Scatton et al., 1983; Miyawaki et al., 1997), the neuropathological literature on the status of the DRN in PD is not entirely clear. In fact, both loss of 5-HT cells bodies and no modification in their number in PD have been reported (Sawada et al., 1985; Jellinger, 1987; Halliday et al., 1990; Paulus and Jellinger, 1991; Kim et al., 2003). Notwithstanding, in primates, surgical lesion of upper brain stem producing contralateral resting tremor and bradykinesia were associated with reduced homolateral striatal 5-HT (Goldstein et al., 1969). Compelling evidence instead exists about damage of the ascending pathways limited to the nerve terminal of 5-HT neurons in different brain regions of PD patients (Chase and Ng, 1972; Chase, 1974; Chinaglia

et al., 1993). Indeed, postmortem examinations have shown a reduction of up to 50% of 5-HT in some areas of the cortex and the basal ganglia (Scatton et al., 1983; Birkmayer and Riederer 1986; Birkmayer and Birkmayer, 1987). Unlike the preferential loss of DA in the putamen, the caudate is affected more than the putamen by loss of all 5-HT markers: 5-HT (−66%), 5-hydroxy-indolacetic acid (5-HIAA; the major 5-HT metabolite) (−42%), 5-HT transporter (5-HTT) (−56%) and tryptophan hydroxylase (TPH; the marker synthetic enzyme) (−59%) (Kish et al., 2008). Reduced brain levels of all of the key markers for the 5-HT neurotransmitter system provide compelling evidence for a striatal serotonergic abnormality in PD. This evidence has been confirmed by antemortem studies and imaging investigations that showed reduced cerebrospinal fluid levels of 5-HIAA and decreased activity of 5-HTT not only in the caudate nucleus and the putamen but also in the thalamus and medial frontal areas, indicating a pathophysiological involvement of 5-HT in PD (Haapaniemi et al., 2001; Kerenyi et al., 2003; Kim et al., 2003). It is, however, very likely that the degree of serotonergic degeneration depends on the stage of the disease (Scholtissen et al., 2006). Nevertheless, Bjorklund's group recently showed that serotonergic innervation of the striatal complex remains relatively intact in most PD patients (Carta et al., 2007).

5-HT receptors' expression in PD animal models and in patients

Animal models are important tools in experimental medical science to better understand the pathogenesis of human diseases such as PD. However, preclinical research on these animal models has provided inconsistent results, highlighting that these experimental models represent only an imperfect replica of human disorders (Scholtissen et al., 2006).

The first PD animal model developed is the 6-OHDA; this agent selectively disrupts catecholaminergic systems and reproduces specific features of PD in rodents, apparently via oxidative damage (Simola et al., 2007 and references therein).

6-OHDA-lesioned animals have been exploited to test therapeutic approaches for treating functional disturbances observed in this disease and will aid the future development of rational therapeutic strategies. The 6-OHDA lesion can be performed in adult or neonatal rats, producing different changes in behaviour and the neurochemistry of these animals, according to the age of the lesion (Breese et al., 2005).

Although independently of the age in which the SNc is injured, 6-OHDA leads to permanent DA ablation while the serotonergic projection to the striatum remains intact. Contrasting results of the activity of DRN serotonergic neurons in 6-OHDA-lesioned rats exist; an increase in frequency and burst firing activity (Chu et al., 2004; Zhang et al., 2007) or a decrease (–60%) of discharge rate (Guiard et al., 2008) has actually been reported.

Both neonatal and adult lesions actually lead to a 5-HT axonal hyperinnervation within the dorsal striatum (Stachowiak et al., 1984; Breese et al., 1985; Zhou et al., 1991; Molina-Holgado et al., 1994; Mrini et al., 1995; Balcioglu et al., 2003; Maeda et al., 2003). Consistently, striatal 5-HT levels and 5-HTT binding have been reported to be increased in this animal model of PD (Commins et al., 1989; Zhou et al., 1991; Guerra et al., 1997; Mendlin et al., 1999; Balcioglu et al., 2003). Interestingly, it has been proposed that striatal ‘reactive’ serotonergic hyperinnervation in lesioned animals occurs to compensate for the lost function of DAergic terminals and this might ‘mask’ the true extent of the 5-HT loss in PD.

This suggests that 5-HT neurotransmission is impaired and the clarification of the pathophysiological mechanism can provide unique information regarding the treatment of parkinsonism from a point of view that differs from conventional therapy.

In addition to DA depletion, a discrete modification in the regulation of postsynaptic 5-HT receptors in different areas of the basal ganglia circuitry and in other brain areas has been observed in animals and humans (Kienzl et al., 1981; Radja et al., 1993). Therefore, alterations of 5-HT binding constants in PD might reflect an imbalance in serotonergic activity.

5-HT_{1A} binding is not altered in the basal ganglia nuclei of neonatally 6-OHDA-lesioned rats. In contrast, there is a considerable increase in binding for 5-HT_{1B} receptors. The highest increase of 5-HT_{1B} binding sites is observed in the SN (54%), the GP (33%) and the two portions of neostriatum. The most likely explanation for the present increases in the neostriatal, nigral and pallidal regions is therefore an augmented production (up-regulation) of these receptors by the neostriatal projection neurons and concomitant increase of their axonal transport to both territories of projection. In view of its widespread distribution in the neostriatum, it also seemed likely that the neostriatal increase in 5-HT_{1B} binding was somehow related to the DA denervation of this brain region rather than to its subsequent 5-HT hyperinnervation, suggesting a possible role for DA in the regulation of 5-HT receptor expression during ontogenesis. A significant increase in the density of 5-HT_{2C} was also revealed throughout the neostriatum (40%) and in the SN (50%), but unchanged in the GP, as if this up-regulation preferentially involved striatonigral as opposed to striatopallidal neurons (Radja et al., 1993).

Consistently, enhanced responses of spontaneously firing units to iontophoresed 5-HT and both 5-HT_{2C} and 5-HT_{2A} agonists have been demonstrated in the 5-HT-hyperinnervated neostriatum after neonatal 6-OHDA lesion (el Mansari et al., 1994). 5-HT_{2A} binding showed an even greater increase (60%), which was restricted to the rostral half of the neostriatum and also seemed imputable to an up-regulation as heteroreceptors. 5-HT_{2A} receptor expression increases significantly on direct pathway neurons (Laprade et al., 1996; Basura and Walker, 1999), even though 5-HT_{2A} receptors are expressed on both direct and indirect striatal projecting neurons (Ward and Dorsa, 1996).

The intracellular mechanisms that mediate the effects of 5-HT within the neonatal lesioned striatum are poorly understood. Neonatal lesions result in altered expression of preprotachykinin (decrease) and preproenkephalin (increase) in direct and indirect striatal pathway neurons,

respectively (Sivam et al., 1987). It has been shown that 5-HT acting via 5-HT₂ receptors could regulate preprotachykinin expression selectively in direct pathway neurons and ultimately motor function, after neonatal DA depletion (Basura and Walker, 2001). Recently, it has been shown that neonatal but not adult 6-OHDA lesions result in a novel coupling of 5-HT_{2A} receptors to the ERK1/2/MAP kinase pathway, a signalling cascade known to regulate neuronal plasticity that is not typically active in these neurons. Because DA-mediated signalling is redundant after 6-OHDA lesions, 5-HT-mediated stimulation of the ERK1/2/MAP kinase pathway may provide an alternative signalling route allowing the regulation of neuronal gene expression and neuronal plasticity in the absence of DA (Brown and Gerfen, 2006).

In adult rats, destruction of the nigrostriatal DA projection by 6-OHDA has not been found to modify [³H]5-HT binding in the neostriatum (Quirion and Richard, 1987).

Consistently, *in situ* hybridization and autoradiographic radioligand studies from lesioned rats and human postmortem tissue from patients with PD have revealed that striatal 5-HT_{1A} (Numan et al., 1995) and 5-HT_{1B} (Zhang et al., 2008) are not influenced by DA depletion. In the striatum, 5-HT_{2A} receptors appear to be up-regulated (Numan et al., 1995) and 5-HT_{2C} receptors down-regulated (Numan et al., 1995; Zhang et al., 2007) or not affected (Basura and Walker, 1999; Fox and Brotchie, 2000b). Striatal 5-HT_{2A} and 5-HT_{2C} are therefore differently regulated in 6-OHDA-lesioned animals. There is no significant difference in 5-HT_{2C} binding level for control vs. PD tissue in the GPi and the GPe (Fox and Brotchie, 2000b), and in the levels of 5-HT_{2C} mRNA between the intact and 6-OHDA-lesioned hemispheres in the nucleus subthalamicus in rats (Zhang et al., 2007). Conversely, 50% increase in 5-HT_{2C} receptor binding was observed in 6-OHDA-lesioned rats, strictly in accordance with the evidence that 5-HT_{2C} receptor binding in the SNr of age-matched control tissue was less than half that in the SNr of patients with PD (Radja et al., 1993; Fox and Brotchie, 2000b).

This evidence highlights a selective change in the 5-HT_{2C} receptor activity only in the output regions of the basal ganglia. 5-HT_{2C} receptors' up-regulation might be compensatory, being a consequence of a decreased level of 5-HT in these nuclei, thus indicating a role for them in the neuronal mechanisms involved in PD (Fox and Brotchie, 2000b).

It is noteworthy that L-DOPA/benserazide treatment not only reversed the 6-OHDA-induced levels of 5-HT_{2A} mRNA in the striatum but also caused a highly significant reduction in the levels of this receptor. In contrast, 5-HT_{2C} mRNA was not affected by L-DOPA/benserazide treatment (Zhang et al., 2007). It can be concluded from these findings that the regulation of 5-HT_{2A} is highly dependent on alterations in DA levels. In contrast, striatal 5-HT_{2C} appears to be regulated by nigrostriatal cell loss and the reduced level(s) of factor(s), other than DA, such as brain-derived neurotrophic factor (BDNF) and cholecystokinin, which are normally expressed in nigrostriatal neurons. Similarly, numerous studies conducted in patients with PD and in animal models of this disease, such as 6-OHDA-lesioned rats and MPTP-treated primates, have shown that L-DOPA-treatment does not adequately reverse the effects of DA cell loss but rather creates a new functional and neurochemical state, which differs both from the normal and lesioned states (Bezard et al., 2003). The fact that 5-HT_{2A}, but not 5-HT_{2C}, receptors are responsive to L-DOPA treatment predicts that pharmacological manipulations at 5-HT_{2C}, but not at 5-HT_{2A}, will result in similar effects in PD patients whether they are treated or not with DA replacement. These data therefore support the notion that 5-HT_{2C} receptor antagonists may be useful as an adjuvant treatment to DA agonists to treat motor complications of PD (Fox et al., 1998; Di Giovanni et al., 2006a; Zhang et al., 2007).

Consistent with these findings, systemic administration of the selective 5-HT_{2C} antagonist SB 206553 was shown to enhance the action of the anti-parkinsonian action of the DA D₁ and D₂ agonists in 6-OHDA-lesioned rats (Fox et al., 1998; Fox and Brotchie, 2000a), suggesting that the use of

a 5-HT_{2C} receptor antagonist in combination with a DA receptor agonist may reduce the reliance upon DA replacement therapies. Hitherto, no studies have been conducted in either non-human primates or humans to address this issue.

5-HT₃ binding is reduced in the entorhinal and prefrontal cortices on the 6-OHDA-lesioned side of the rat brain while no changes in the amygdala and the hippocampus were observed (Cicin-Sain and Jenner, 1993). Unfortunately, these authors did not measure the 5-HT₃ binding in the basal ganglia. Nevertheless, it has been suggested that the 5-HT₃ blockade might be important in the effect of the anti-parkinsonian agent talipexole (Nishio et al., 1996).

No modification of the distribution and density of 5-HT₄ receptor binding sites was observed in 6-OHDA-lesioned guinea pig basal ganglia — neither in the caudate-putamen nor in the SN itself (Vilario et al., 2005). On the other hand, following lesion of DA neurons by intranigral injection of 6-OHDA, an increased 5-HT₄ receptor binding was instead observed in the caudal (59%), but not the rostral, part of the caudate-putamen as well as in the GP (93%) (Compan et al., 1996). Since no decreases in 5-HT₄ receptor density have been detected in both studies (Compan et al., 1996; Vilario et al., 2005) after the DA lesion, it is likely that these receptors are not expressed in DA neurons but are located on terminals of striatal projection neurons. Kainic acid lesions of the caudate-putamen were associated with dramatic local decreases in 5-HT₄ receptor binding on the injected side (–89%), which suggested that striatal neurons expressed 5-HT₄ receptors. Corresponding decreases of 72 and 20% in receptor density were detected in the GP and the SN, consistent with a presumed localization of 5-HT₄ receptors on striatal GABA neurons projecting to these regions. In the SN, the decrease in [³H]GR 113808 binding was localized to the pars lateralis, indicating that striatal neurons belonging to the cortico-striato-nigro-tectal pathway and containing GABA and dynorphin express 5-HT₄ receptors. As yet, no experimental evidence has been found about the modification in expression of 5-HT₅, 5-HT₆ and 5-HT₇ receptors in the basal ganglia of an animal model of PD.

Role of 5-HT in LID

The abnormal involuntary movements, or dyskinesia, generated by prolonged administration of L-DOPA represent one of the major challenges facing current therapy for PD. These debilitating motor disturbances are all the more problematic because L-DOPA, in spite of its introduction several decades ago, still represents the therapy of choice for the treatment of PD. The discovery of pharmacological interventions able to counteract LID would therefore represent an important breakthrough in the therapy for PD. The design of novel agents for the prevention and treatment of LID requires the elucidation of the adaptive changes produced in the parkinsonian brain by repeated administration of L-DOPA and the assessment of their role in the development and expression of this condition. Recently, compelling evidence has been produced about the causative role of 5-HT in LID developing in both animal models and PD patients, suggesting a use of serotonergic agents in reducing LID in PD patients (Jackson et al., 2004; Johnston and Brotchie, 2006; Carlsson et al., 2007; Carta et al., 2007). In fact, 5-HT neurons have been shown to be able to convert exogenous L-DOPA to DA, and store and release DA in an activity-dependent manner but without the fine control that occurs in DA release by DA neurons (see Chapter 22). Therefore, activation of the autoreceptors 5-HT_{1A} and 5-HT_{1B}, localized on the soma and terminals of 5-HT neurons, respectively, has been shown to be highly effective in counteracting LIDs in the 6-OHDA rat model (Carta et al., 2007). The rationale for the use of these agonists consists in a possible modulation of DA release from 5-HT neurons in a way that resembles the physiological DA release from DA neurons. Hitherto, this attractive hypothesis has not been validated by a human study.

In addition, 5-HT_{2C} receptors might be involved in LID. The changes in 5-HT_{2C} receptor binding reported by Fox et al. (1998) were seen in PD patients with LID. It is thus possible that they could be ascribed to the process underlying dyskinesia rather than parkinsonism (Fox et al., 1998). Thus, reduced stimulation of 5-HT_{2C}

receptors would lead to decreased activity of the basal ganglia output nuclei and increased levels of abnormal movements. This hypothesis may predict an efficacious use of 5-HT_{2C} agonists for alleviating the side effects of long-term treatment with L-DOPA. This seems unlikely since clozapine and quetiapine, antagonists for this receptor subtype, have been used successfully for this purpose (Durif et al., 2004). On the other hand, systemic administration of the selective 5-HT_{2C} antagonist SB 206553 was shown to enhance the anti-parkinsonian action of the DA D₁ and D₂ agonists in 6-OHDA-lesioned rats (Fox et al., 1998; Fox and Brotchie, 2000a), suggesting that the use of a 5-HT_{2C} receptor antagonist in combination with a DA receptor agonist may reduce the reliance upon DA replacement therapies. Hitherto, no studies have been conducted in either non-human primates or humans to address this issue. For a more extended treatment of this subject, see Chapters 22 and 23.

Role of 5-HT in parkinsonian resting tremor

Furthermore, convincing evidence has indicated a pivotal role of 5-HT in parkinsonian resting tremor. The most common tremor seen in patients with PD is a ‘pill-rolling’ movement of the hands (Sethi, 2003). Despite a number of clinical and basic studies, the neural substrate for this motor complication remains unclear. The causative role of 5-HT in tremorgenesis has been strengthened by a recent PET study in PD patients (Doder et al., 2003). Interestingly, these authors showed that severity of parkinsonian tremor, but not rigidity or bradykinesia, was correlated significantly with this decrease in midbrain raphe 5-HT_{1A} binding, likely reflecting a dysfunction and loss of serotonergic cell bodies early in the disease process (Doder et al., 2003). This evidence is in agreement with the suggested hypothesis of ‘disequilibria’ in the 5-HT–histamine system responsible for tremor (akathisia), whereas ‘disequilibria’ in the DA–ACh system might lead to rigidity (akinesia) (Barbeau, 1962). A glimmer of light on the potential of subtype-selective serotonergic agents for the relief of parkinsonian tremor was recently thrown by Carlson and colleagues (Carlson et al., 2003).

Local injections of a mixed 5-HT_{2A/2C} receptor antagonist into the SNr block tremulous jaw movements in a cholinomimetic model of parkinsonian tremor in rats (Carlson et al., 2003). This result is consistent with previous studies showing that the jaw movement activity was suppressed potently by clozapine, olanzapine and risperidone (Ikeguchi and Kuroda, 1995; Trevitt et al., 1997, 1998) and clinical reports demonstrating serotonergic involvement in the generation and treatment of parkinsonian symptoms and other motor disorders (Ikeguchi and Kuroda, 1995).

Role of 5-HT in psychiatric complications in PD

Besides being a movement disorder, PD is also associated with numerous non-motor symptoms. Mood disturbance, and especially major depressive disorder, has an average prevalence of 25–40% in outpatient settings (Leentjens, 2004; Veazey et al., 2005; Miller et al., 2007). According to the serotonergic hypothesis of depression in PD (Mayeux, 1990), 5-HT seems to play a central role. Indeed, it has been suggested that the reduced DA activity in PD can lead, as physiological adaptation, to a reduction of serotonergic tone and, at the same time, constitute a risk factor for depression (Mayeux, 1990). The presence of this biological risk factor for depression may explain the high prevalence of this condition in patients with PD (Leentjens, 2004). Nevertheless, a recent double-blind, randomized study provided no support for the serotonergic hypothesis of depression in PD, using the acute tryptophan depletion (ATD) paradigm (Leentjens et al., 2006). Comorbid depression in PD is usually treated with SSRIs or tricyclic antidepressants (TCAs), although these drugs should be used with caution in patients with PD because they can exacerbate orthostatic hypotension and anticholinergic adverse effects (Veazey et al., 2005).

We have recently suggested that 5-HT_{2C} receptor antagonists prove useful in addressing depression and also, in PD patients, increasing the activity of ventral tegmental area (VTA) DA neurons and accumbal DA release (Di Giovanni et al., 2006a, b). In accord with this, nefazodone, a 5-HT₂ antagonist/reuptake inhibitor, has shown a

dual activity: as an antidepressant and as an agent capable of reducing the EPS in depressed PD patients (Avila et al., 2003). Given these desirable motor side effects, nefazodone should be chosen over SSRIs for the treatment of depression in PD patients if they tolerate its use (Avila et al., 2003).

Apart from depression, dementia and psychosis are also common psychiatric problems associated with PD. Psychotic complications are associated with the use of anti-parkinsonian drugs and make PD management more difficult, given the need for anti-DAergic therapy, which worsens motor functioning in patients with PD. For psychosis, clozapine is the only atypical antipsychotic that has proven effective without worsening motor function in PD patients. However, its use requires the monitoring of agranulocytosis. Newer atypical APDs, such as quetiapine, have been claimed to be safe in terms of motor functioning, but evidence about their effectiveness is not compelling. The efficacy of the atypical APDs in treating psychosis in PD patients is likely to be due to their ability in blocking 5-HT receptors. In fact, antagonists or inverse agonists of the 5-HT_{2A} and 5-HT_{2C} receptor are potential therapeutic agents for treatment-induced psychosis of PD (Weiner et al., 2003; Di Giovanni et al., 2006a).

Role of 5-HT in APD-induced extrapyramidal side effects

In addition to the neurodegenerative, idiopathic form, parkinsonian symptoms are also produced by therapies using typical APDs such as haloperidol, a mixed DA antagonist. In fact, EPS are common neurological side effects of APD medication. More than 60% of the people who take conventional antipsychotic medications experience some form of EPS. These side effects can occur within the first few days or weeks of treatment or appear after months and years of antipsychotic medication use. EPS are more common among patients taking conventional antipsychotic medications, compared to the newer atypical drugs. EPS can cause a variety of symptoms, e.g. involuntary movements, tremors and rigidity, body restlessness, muscle contractions and changes

in breathing and heart rate. Drug-induced EPS are categorized into acute (acute dystonia, parkinsonism and akathisia) and delayed [tardive dyskinesia (TD)] syndromes, based on the time of occurrence during antipsychotic treatment. Neuroleptic-induced parkinsonism is characterized by the triad of tremor, rigidity and bradykinesia; it can closely resemble idiopathic PD caused by nigrostriatal degeneration. TD is the major limitation of long-term APD therapy, being potentially irreversible. This condition occurs in 15–30% of patients and results in abnormal, unintentional choreoathetoid movements of the head, limbs and trunk. The incidence of akathisia, parkinsonian syndromes and TD, which are relatively frequent in patients treated with classical antipsychotic agents, is also similar to that in patients treated with TCAs and SSRIs. Amitriptyline, clomipramine, doxepine, trazodone and fluoxetine induce TD in patients not previously treated with neuroleptics (Mander et al., 1994; Clayton, 1995; Bharucha and Sethi, 1996), and akathisia, acute dystonia and pseudo-parkinsonism have been induced by some of these drugs, although not all share the same risk for the development of movement disturbances.

It has been proposed that DA receptors' supersensitivity, arising from up-regulation of DA₂ receptors following APDs, is the base for the development of TD. In addition to DA, 5-HT receptors are also important in the aetiology of schizophrenia and the elicitation of EPS. Particularly 5-HT_{1A}, 5-HT_{2A/2C} and 5-HT₃ receptor signalling seems to be altered in TD. Recently, a possible genetic predisposition to develop TD exists, and association between TD and receptor gene polymorphisms of 5-HT_{2A} and 5-HT_{2C} genes has actually been shown (Gunes et al., 2007, 2008).

5-HT_{1A} receptors may be important since the postsynaptic 5-HT_{1A} receptor density in the prefrontal and temporal cortices of patients with schizophrenia is elevated (Tauscher et al., 2002). Probably, a resultant decrease in the normal inhibitory serotonergic influence on motor activity may be involved in the precipitation of TD in patients on haloperidol therapy, since chronic administration of haloperidol increased the responsiveness of presynaptic and postsynaptic 5-HT_{1A} receptors and reduced 5-HT turnover

(Haleem and Khan, 2003). Consistently, repeated administration of low doses of buspirone, a partial agonist at 5-HT_{1A} receptors, decreased the responsiveness of 5-HT_{1A} receptors (Okazawa et al., 1999) and reversed haloperidol-induced deficits of exploratory activity (Haleem et al., 2007a, b). In a widely accepted rat model of TD obtained with chronic treatment by neuroleptics (Ellison and See, 1989) 8-OHDPAT (Naidu and Kulkarni, 2001a) and sarizotan, a 5-HT_{1A} agonist/D₃/D₄ ligand (Rosengarten et al., 2006) treatment inhibited haloperidol-induced vacuous chewing movements (VCMs). Consistently, buspirone reversed reserpine-induced dyskinetic movements in rats (Queiroz and Frussa-Filho, 1999) and completely reversed the induction of tardive VCMs (Haleem et al., 2007a, b).

The evidence that buspirone, at low doses, preferentially acts at the somatodendritic 5-HT_{1A} receptors further supports the hypothesis of impaired somatodendritic 5-HT function (Haleem and Khan, 2003; Haleem et al., 2007a, b). These compelling experimental data support an impairment of 5-HT transmission as a possible important contributing factor in the onset of TD and other parkinsonian-like effects of neuroleptics. Repeated administration of haloperidol elicits an increase in the responsiveness of somatodendritic and post-synaptic 5-HT_{1A} receptors. An increase in the effectiveness of somatodendritic 5-HT_{1A} receptors in rats treated with haloperidol would be expected to decrease the 5-HT input to DA nuclei. Buspirone and 8-OHDPAT might act in reversal of haloperidol-induced dyskinesia by desensitization of 5-HT_{1A} receptors, suggesting that prior administration of buspirone may be of help in the improvement of EPS induced by haloperidol and other antipsychotic drugs (Haleem et al., 2004; Samad et al., 2007).

5-HT_{2C} receptor signalling is also deeply implicated in the development of VCMs and 5-HT_{2C} receptors, which become supersensitized during long-term haloperidol treatment and remain supersensitized even after withdrawal of haloperidol as a treatment (Wolf et al., 2005; Ikram et al., 2007). In fact, mCPP enhances the oral activity response to a greater extent than the DA D₁ receptor agonist SKF 38393 in neonatal 6-OHDA-treated

rats (Gong and Kostrzewa, 1992). It is likely that mCPP produces its effect at 5-HT₂ (probably 5-HT_{2C}) receptors being blocked by the largely 5-HT₂ receptor antagonist mianserin (Gong and Kostrzewa, 1992). Moreover, DA D₁-induced VCMs also appear to be mediated ultimately by 5-HT_{2C} receptors and, as a result, are attenuated by mianserin (Gong and Kostrzewa, 1992). It is significant that various 5-HT₂ receptor antagonists, such as seganserin, ketanserin and ritanserin, are able to effectively reduce the number of spontaneous VCMs in a rat model of TD (Naidu and Kulkarni, 2001a).

Concordantly, clozapine and other atypical APDs that also have a high affinity for 5-HT_{2C} receptors (Altar et al., 1986) reduced motor side effect liability because of the same degree of intrinsic anti-parkinsonian characteristics, which act to counteract the pro-parkinsonian effects of DA blockade. This was suggested by early clinical studies indicating that the 5-HT_{2A/2C} receptor antagonist ritanserin (Leysen et al., 1985; Bersani et al., 1990) can ameliorate negative symptoms as well as attenuate exciting EPS in schizophrenics treated with classical APDs (Bersani et al., 1990; Miller et al., 1990). The relevance of 5-HT_{2C} receptor blocking in the effect of atypical APDs was shown by Canton et al. (1990), who revealed the high affinity of clozapine and risperidone for 5-HT_{2C} sites in the rat choroid plexus. These findings were subsequently confirmed (Kuoppamaki et al., 1993, 1995; Schotte et al., 1993; Canton et al., 1994) and extended to brain sections (Roth et al., 1992, 1998). Antagonism at 5-HT_{2C} receptors by several atypical antipsychotics was also observed in vivo. Indeed, clozapine produces an increase in extracellular levels of DA in the nucleus accumbens (Di Matteo et al., 2002; Shilliam and Dawson, 2005), reverses the inhibition of accumbal DA release induced by the 5-HT_{2C} agonist Ro 60-175 (Di Matteo et al., 2002) and blocks the hypolocomotion induced by the 5-HT_{2C} agonist mCPP (Prinssen et al., 2000).

It is noteworthy that clozapine, like several atypical APDs, behaves as a 5HT_{2C} inverse agonist in heterologous expression systems in vitro (Herrick-Davis et al., 2000; Rauser et al., 2001;

Navailles et al., 2006) and in vivo (Navailles et al., 2006). Thus, the 5-HT_{2C} receptor inverse agonist might underlie the unique clinical properties of atypical APDs, such as low EPS profile and good anti-dyskinetic efficacy (Herrick-Davis et al., 2000; Durif et al., 2004; Navailles et al., 2006).

Despite different chemical structures and pharmacodynamic signalling pathways, a number of antipsychotics inhibit ion fluxes through 5-HT₃ receptors in a noncompetitive manner, with the exception of the known competitive antagonists mirtazapine, olanzapine and clozapine (Bymaster et al., 2001; Rammes et al., 2004; Eisensamer et al., 2005). In accord with these findings, it has been shown that 5-HT₃ receptor antagonists ondansetron and tropisetron dose dependently reversed the haloperidol-induced VCMs and reduced haloperidol-induced wet dog shakes, further supporting the possibility of an alteration in the serotonergic system after chronic haloperidol treatment (Naidu and Kulkarni, 2001b). It can be concluded that these 5-HT receptors can serve as potential therapeutic targets for the development of novel molecules for the treatment and prevention of TD.

Catalepsy

The neuroleptic-induced state of catalepsy is generally considered as an animal model of the akinesia and rigidity seen in PD, which is predictive of EPS for APDs. 5-HT is also implicated in APD-induced catalepsy (Wadenberg, 1996). For example, different SSRIs attenuate haloperidol-induced catalepsy in mice (Pires et al., 2005). In addition, the 5-HT_{1A} receptor agonists 8-OHDPAT and buspirone, and the 5-HT_{2A/2C} receptor agonists DOI and 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane (DOB), attenuate D₂ receptor-mediated catalepsy (Invernizzi et al., 1988; Hicks, 1990; Neal-Beliveau et al., 1993; Lucas et al., 1997). Moreover, the anti-cataleptic effects of 8-OHDPAT and DOI are antagonized by WAY 100635, a selective 5-HT_{1A} receptor antagonist (Bartoszyk et al., 1996), and mianserin, a 5-HT_{2A/2C} receptor antagonist (Neal-Beliveau et al., 1993), respectively. In addition, 5-HT_{1A} receptor activation reduces the cataleptogenic

potential of novel antipsychotic agents such as ziprasidone, aripiprazole, bifeprunox, SLV313, SSR181507 and sarizotan (Kleven et al., 2005). These findings strongly indicate that the serotonergic system is involved in the onset of catalepsy. There is strong evidence to suggest that (1) the catalepsy produced by DA D₁ or D₂ receptor antagonists can be completely antagonized by the administration of 5-HT_{1A} receptor agonists acting at 5-HT_{1A} autoreceptors in the DRN and (2) the catalepsy produced by a DA D₂ receptor antagonist can be completely antagonized by treatment with a 5-HT_{2A/2C} receptor agonist.

On the other hand, it has been shown that clozapine inhibits the cataleptic response to loxapine and olanzapine but does not induce catalepsy by itself (Kalkman et al., 1997). In addition, behavioural evidence shows that SB 228357, a selective 5-HT_{2B/2C} receptor antagonist, and ACP-103, a 5-HT_{2A} receptor inverse agonist, attenuated catalepsy produced by haloperidol or risperidone (Reavill et al., 1999; Gardell et al., 2007). On the other hand, the 5-HT_{2A} and 5-HT_{2B} receptor antagonists MDL 100907 and SB 215505 did not reverse haloperidol-induced catalepsy. These data suggest a role for 5-HT_{2C} receptors in the anti-cataleptic action of SB 228357. Thus, the blockade of 5-HT_{2C} receptors, instead of their activation, would play a role in relieving the neuroleptic EPS disturbance. This hypothesis is further reinforced by the observation that the 5-HT_{2C} receptor activation by Ro 600175 per se induces catalepsy (Grottick et al., 2000).

Therefore, another interesting application of the data regarding the functional role of 5-HT in the basal ganglia is the possible use of 5-HT receptor ligands in the treatment of drug-induced parkinsonian syndromes in relation to present efforts to develop new atypical neuroleptics with affinity for brain 5-HT receptor subtypes.

Hitherto, only one piece of research has investigated the effect of 5-HT₆ receptor antagonism on catalepsy and in rats with unilateral 6-OHDA lesions (Bourson et al., 1998). Ro 04-6790, a 5-HT₆ selective antagonist, did not induce catalepsy nor did it have any effect on either haloperidol- or SCH 23390-induced catalepsy. Administration of Ro 04-6790 was instead

able to reverse rotational behaviour induced by the muscarinic antagonists scopolamine and atropine in unilaterally 6-OHDA-lesioned rats. These data suggest that the 5-HT₆ receptor is involved in the control of cholinergic neurotransmission in the striatum that is distal to DA transmission. A possible mechanism could be an increase in ACh release induced by 5-HT₆ receptor blockades directly or through modulation of GABA neurotransmission in the striatum, since 5-HT₆ receptors are expressed on GABA spiny neurons. In addition, Ro 04-6790 alone did not induce turning behaviour in the unilaterally lesioned rat nor did it potentiate or inhibit ipsilateral rotations induced by amphetamine. Furthermore, Ro 04-6790 had no effect on the contralateral rotations induced by L-DOPA. Therefore, 5-HT₆ receptor affinity does not account for the lack of EPS — effects characteristic of clozapine-like compounds (Bourson et al., 1998).

Conclusions

From the large amount of literature reviewed here, it appears evident that the serotonergic neurotransmitter system plays a pivotal role in the modulation of basal ganglia circuitry (Fig. 1), and its dysfunction is involved in the pathophysiology of PD and other motor disorders.

Among all the 5-HT receptors present in the basal ganglia nuclei, the 5-HT_{1A/1B} and 5-HT_{2A/2C} receptors are particularly important, because of their localization and regulatory role on neurotransmitter release in many basal ganglia circuitry, including GLUergic terminals in the SNc (Pineyro and Blier, 1999). 5-HT_{1A} receptor agonists have the potential for being useful at different stages of PD progression since they may provide symptomatic relief in early PD, and anti-dyskinetic efficacy in a later stage. Moreover, it has been shown (Bezard et al., 2006) that 5-HT_{1A} receptor agonists could also present (partial) neuroprotection, partially blocking the excitotoxic cycle of circuit-driven degeneration caused by the overactivity of the STN afferents of DA neurons, decreasing the hyperactivity of the remaining DA neurons through opening of potassium

conductances and suppressing activity of the vulnerability factor caspase-3 in the remaining DA neurons.

Although several selective agents for 5-HT receptors have been discovered, none has reached the market for the treatment of motor disorders as yet. However, several companies are very active in 5-HT receptor research in PD, although they have concentrated on different receptor subtypes. Nevertheless despite the promising findings, last year, Merck KGaA decided to stop further development of its late-stage development drug sarizotan as a treatment for PD. The company took the decision after examining the results of two Phase III studies of sarizotan in advanced PD patients with dyskinesia. A statement from Merck KGaA said that the Phase III studies did not confirm earlier Phase II findings or the results from preclinical studies. The two trials, PADDY 1 and PADDY 2, investigated twice-daily dosing of sarizotan 1-mg tablets, to investigate whether the drug could achieve a 25% improvement in dyskinesia symptoms, but it failed to meet this efficacy threshold.

From the data reviewed here, it should be clear that drugs acting at the 5-HT_{2A/2C} receptors might be an important feature of the treatment of PD, drug-related motor disturbances and the psychiatric symptoms often associated with these neurological disorders. Nevertheless, as happened for the 5-HT_{1A} receptors, no compounds selective for 5-HT_{2A/2C} receptors have been released for the treatment of major motor disturbances such as PD. A glimmer of hope comes from the pharmaceutical company Acadia, which, in 2007, initiated the first Phase III pivotal trial with pimavanserin, a 5-HT_{2A} inverse agonist in PD patients with PD psychosis, after having positively concluded Phase II.

NS-2330, a triple monoamine reuptake inhibitor, has shown therapeutic potential in PD. In 2006, NeuroSearch's global partner Boehringer Ingelheim has concluded that the results from three completed Phase II clinical studies in PD did not meet the company's efficacy criteria to proceed with Phase III clinical development.

5-HT research is now more than 50 years old and has generated a wealth of therapeutic agents,

some of which have had a major impact on disease management. SSRIs are among the most widely prescribed drugs for treating depression and a variety of other disorders, including anxiety and social phobia. But we are a long way from a serotonergic therapeutic intervention for PD.

5-HT receptor research has generated detailed information on the molecular biology and regional and cellular localization of these receptors. A major challenge now is to utilize this knowledge to develop receptor-specific drugs and use the information gained to better treat CNS disorders. In addition, further clarification of the role of 5-HT transmission in the pathophysiology of basal ganglia disorders is required since the overall picture is still confusing. Furthermore, most of the data came from animal studies and animal models of PD that yielded contrary results compared to clinical studies. This leaves room for speculation about the real value of preclinical research for clinical PD. Moreover, there are also many avenues that remain unexplored, so there are undoubtedly further advances to be made.

Abbreviations

5-HIAA	5-hydroxy-indolacetic acid
5-HT	serotonin
5-HTT	5-HT transporter
6-OHDA	6-hydroxydopamine
ACh	acetylcholine
AHPs	after-hyperpolarizations
APDs	antipsychotic drugs
ATD	acute tryptophan depletion
CNS	central nervous system
DA	dopamine
DBS	deep brain stimulation
DRN	dorsal raphe nucleus
EPN	entopeduncular nucleus
EPS	extrapyramidal symptoms
EPSCs	excitatory postsynaptic currents
GLU	glutamate
GP	globus pallidus
GPe	external segment of the GP

GPI	internal segment of the GP
HFS	high-frequency stimulation
IPSCs	inhibitory postsynaptic currents
LAIs	large aspiny interneurons
L-DOPA	levodopa
LID	L-DOPA-induced dyskinesia
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MRN	medial raphe nucleus
MSNs	medium spiny neurons
NMR	nucleus raphe magnus
NRO	nucleus raphe obscurus
NRP	nucleus raphe pallidus
PLC	phospholipase C
PPN	pedunculopontino nucleus
SN	substantia nigra
SNc	SN pars compacta
SNr	SN pars reticulata
SSRIs	selective serotonin reuptake inhibitors
STN	subthalamic nucleus
TCAs	tricyclic antidepressants
TD	tardive dyskinesia
TPH	tryptophan hydroxylase
VCMs	vacuous chewing movements
VTA	ventral tegmental area

References

- Abi-Dargham, A., Laruelle, M., Wong, D.T., Robertson, D.W., Weinberger, D.R. and Kleinman, J.E. (1993) Pharmacological and regional characterization of [3H]LY278584 binding sites in human brain. *J. Neurochem.*, 60(2): 730–737.
- Albin, R.L., Young, A.B. and Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.*, 12(10): 366–375.
- Altar, C.A., Wasley, A.M., Neale, R.F. and Stone, G.A. (1986) Typical and atypical antipsychotic occupancy of D2 and S2 receptors: an autoradiographic analysis in rat brain. *Brain Res. Bull.*, 16(4): 517–525.
- Antonelli, T., Fuxe, K., Tomasini, M.C., Bartoszyk, G.D., Seyfried, C.A., Tanganelli, S. and Ferraro, L. (2005) Effects of sarizotan on the corticostriatal glutamate pathways. *Synapse*, 58(3): 193–199.
- Avila, A., Cardona, X., Martin-Baranera, M., Maho, P., Sastre, F. and Bello, J. (2003) Does nefazodone improve both depression and Parkinson disease? A pilot randomized trial. *J. Clin. Psychopharmacol.*, 23(5): 509–513.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal

- and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179(3): 641–667.
- Balcioglu, A., Zhang, K. and Tarazi, F.I. (2003) Dopamine depletion abolishes apomorphine- and amphetamine-induced increases in extracellular serotonin levels in the striatum of conscious rats: a microdialysis study. *Neuroscience*, 119(4): 1045–1053.
- Barbeau, A. (1962) The pathogenesis of Parkinson's disease: a new hypothesis. *Can. Med. Assoc. J.*, 87: 802–807.
- Barnes, J.M., Barnes, N.M., Champaneria, S., Costall, B. and Naylor, R.J. (1990) Characterisation and autoradiographic localisation of 5-HT₃ receptor recognition sites identified with [3H]-(S)-zacopride in the forebrain of the rat. *Neuropharmacology*, 29(11): 1037–1045.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Bartoszyk, G.D., Roos, C. and Ziegler, H. (1996) 5-HT_{1A} receptors are not involved in clozapine's lack of cataleptogenic potential. *Neuropharmacology*, 35(11): 1645–1646.
- Barwick, V.S., Jones, D.H., Richter, J.T., Hicks, P.B. and Young, K.A. (2000) Subthalamic nucleus microinjections of 5-HT₂ receptor antagonists suppress stereotypy in rats. *Neuroreport*, 11(2): 267–270.
- Basura, G.J. and Walker, P.D. (1999) Serotonin 2A receptor mRNA levels in the neonatal dopamine-depleted rat striatum remain upregulated following suppression of serotonin hyperinnervation. *Brain Res. Dev. Brain Res.*, 116(1): 111–117.
- Basura, G.J. and Walker, P.D. (2001) Serotonin 2A receptor regulation of striatal neuropeptide gene expression is selective for tachykinin, but not enkephalin neurons following dopamine depletion. *Brain Res. Mol. Brain Res.*, 92(1–2): 66–77.
- Bata-Garcia, J.L., Heredia-Lopez, F.J., Alvarez-Cervera, F.J., Arankowsky-Sandoval, G. and Gongora-Alfaro, J.L. (2002) Circling behavior induced by microinjection of serotonin reuptake inhibitors in the substantia nigra. *Pharmacol. Biochem. Behav.*, 71(1–2): 353–363.
- Belforte, J.E. and Pazo, J.H. (2004) Turning behaviour induced by stimulation of the 5-HT receptors in the subthalamic nucleus. *Eur. J. Neurosci.*, 19(2): 346–355.
- Bengtson, C.P., Lee, D.J. and Osborne, P.B. (2004) Opposing electrophysiological actions of 5-HT on noncholinergic and cholinergic neurons in the rat ventral pallidum in vitro. *J. Neurophysiol.*, 92(1): 433–443.
- Bersani, G., Grisipini, A., Marini, S., Pasini, A., Valducci, M. and Ciani, N. (1990) 5-HT₂ antagonist ritanserin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. *Clin. Neuropharmacol.*, 13(6): 500–506.
- Bezard, E., Brotchie, J.M. and Gross, C.E. (2001) Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat. Rev. Neurosci.*, 2(8): 577–588.
- Bezard, E., Gerlach, I., Moratalla, R., Gross, C.E. and Jork, R. (2006) 5-HT_{1A} receptor agonist-mediated protection from MPTP toxicity in mouse and macaque models of Parkinson's disease. *Neurobiol. Dis.*, 23(1): 77–86.
- Bezard, E., Gross, C.E. and Brotchie, J.M. (2003) Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. *Trends Neurosci.*, 26(4): 215–221.
- Bharucha, K.J. and Sethi, K.D. (1996) Complex movement disorders induced by fluoxetine. *Mov. Disord.*, 11(3): 324–326.
- Bhidayasiri, R. and Truong, D.D. (2004) Chorea and related disorders. *Postgrad. Med. J.*, 80(947): 527–534.
- Birkmayer, W. and Birkmayer, J.D. (1987) Dopamine action and disorders of neurotransmitter balance. *Gerontology*, 33(3–4): 168–171.
- Birkmayer, W. and Riederer, P. (1986) Biological aspects of depression in Parkinson's disease. *Psychopathology*, 19(2): 58–61.
- Bishop, C., Daut, G.S. and Walker, P.D. (2005) Serotonin 5-HT_{2A} but not 5-HT_{2C} receptor antagonism reduces hyperlocomotor activity induced in dopamine-depleted rats by striatal administration of the D₁ agonist SKF 82958. *Neuropharmacology*, 49(3): 350–358.
- Bishop, C., Tessmer, J.L., Ullrich, T., Rice, K.C. and Walker, P.D. (2004) Serotonin 5-HT_{2A} receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT_{2A/2C} agonism. *J. Pharmacol. Exp. Ther.*, 310(2): 687–694.
- Blackburn, T.P. (2004) Serotonergic agents and Parkinson's disease. *Drug Discov. Today Ther. Strateg.*, 1: 35–41.
- Blackburn, T.P., Cox, B., Heapy, C.G., Lee, T.F. and Middlemiss, D.N. (1981) Supersensitivity of nigral serotonin receptors and rat rotational behaviour. *Eur. J. Pharmacol.*, 71(2–3): 343–346.
- Blackburn, T.P., Kemp, J.D., Martin, D.A. and Cox, B. (1984) Evidence that 5-HT agonist-induced rotational behaviour in the rat is mediated via 5-HT₁ receptors. *Psychopharmacology (Berl.)*, 83(2): 163–165.
- Blomeley, C. and Bracci, E. (2005) Excitatory effects of serotonin on rat striatal cholinergic interneurons. *J. Physiol. (Lond.)*, 569(Pt 3): 715–721.
- Blurton, P.A. and Wood, M.D. (1986) Identification of multiple binding sites for [3H]5-hydroxytryptamine in the rat CNS. *J. Neurochem.*, 46(5): 1392–1398.
- Boess, F.G. and Martin, I.L. (1994) Molecular biology of 5-HT receptors. *Neuropharmacology*, 33(3–4): 275–317.
- Bonaventure, P., Hall, H., Gommeren, W., Cras, P., Langlois, X., Jurzak, M. and Leysen, J.E. (2000) Mapping of serotonin 5-HT₄ receptor mRNA and ligand binding sites in the post-mortem human brain. *Synapse*, 36(1): 35–46.
- Bonaventure, P., Voorn, P., Luyten, W.H., Jurzak, M., Schotte, A. and Leysen, J.E. (1998) Detailed mapping of serotonin 5-HT_{1B} and 5-HT_{1D} receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function. *Neuroscience*, 82(2): 469–484.
- Bonsi, P., Cuomo, D., Ding, J., Sciamanna, G., Ulrich, S., Tschert, A., Bernardi, G., Surmeier, D.J. and Pisani, A. (2007a) Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT_{2C}, 5-HT₆, and 5-HT₇ serotonin receptors: implications for extrapyramidal

- side effects of serotonin reuptake inhibitors. *Neuropsychopharmacology*, 32(8): 1840–1854.
- Bonsi, P., Cuomo, D., Picconi, B., Sciamanna, G., Tschertter, A., Tolu, M., Bernardi, G., Calabresi, P. and Pisani, A. (2007b) Striatal metabotropic glutamate receptors as a target for pharmacotherapy in Parkinson's disease. *Amino Acids*, 32(2): 189–195.
- Boschert, U., Amara, D.A., Segu, L. and Hen, R. (1994) The mouse 5-hydroxytryptamine_{1B} receptor is localized predominantly on axon terminals. *Neuroscience*, 58(1): 167–182.
- Bourson, A., Boess, F.G., Bös, M. and Sleight, A.J. (1998) Involvement of 5-HT₆ receptors in nigro-striatal function in rodents. *Br. J. Pharmacol.*, 125(7): 1562–1566.
- Breese, G.R., Baumeister, A., Napier, T.C., Frye, G.D. and Mueller, R.A. (1985) Evidence that D-1 dopamine receptors contribute to the supersensitive behavioral responses induced by L-dihydroxyphenylalanine in rats treated neonatally with 6-hydroxydopamine. *J. Pharmacol. Exp. Ther.*, 235(2): 287–295.
- Breese, G.R., Knapp, D.J., Criswell, H.E., Moy, S.S., Papadeas, S.T. and Blake, B.L. (2005) The neonate-6-hydroxydopamine-lesioned rat: a model for clinical neuroscience and neurobiological principles. *Brain Res. Brain Res. Rev.*, 48(1): 57–73.
- Brodie, B.B., Pletscher, A. and Shore, P.A. (1955) Evidence that serotonin has a role in brain function. *Science*, 122(3177): p. 968.
- Brown, P. and Gerfen, C.R. (2006) Plasticity within striatal direct pathway neurons after neonatal dopamine depletion is mediated through a novel functional coupling of serotonin 5-HT₂ receptors to the ERK 1/2 map kinase pathway. *J. Comp. Neurol.*, 498(3): 415–430.
- Bruggeman, R., Heeringa, M., Westerink, B.H. and Timmerman, W. (2000) Combined 5-HT₂/D₂ receptor blockade inhibits the firing rate of SNR neurons in the rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 24(4): 579–593.
- Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D. and Palacios, J.M. (1994) Localization of 5-HT_{1B}, 5-HT_{1D} α , 5-HT_{1E} and 5-HT_{1F} receptor messenger RNA in rodent and primate brain. *Neuropharmacology*, 33(3–4): 367–386.
- Bruinvels, A.T., Palacios, J.M. and Hoyer, D. (1993a) Autoradiographic characterisation and localisation of 5-HT_{1D} compared to 5-HT_{1B} binding sites in rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 347(6): 569–582.
- Bruinvels, A.T., Palacios, J.M. and Hoyer, D. (1993b) 5-hydroxytryptamine₁ recognition sites in rat brain: heterogeneity of non-5-hydroxytryptamine_{1A}/1C binding sites revealed by quantitative receptor autoradiography. *Neuroscience*, 53(2): 465–473.
- Bubser, M., Backstrom, J.R., Sanders-Bush, E., Roth, B.L. and Deutch, A.Y. (2001) Distribution of serotonin 5-HT(2A) receptors in afferents of the rat striatum. *Synapse*, 39(4): 297–304.
- Buften, K.E., Steward, L.J., Barber, P.C. and Barnes, N.M. (1993) Distribution and characterization of the [³H]granisetron-labelled 5-HT₃ receptor in the human forebrain. *Neuropharmacology*, 32(12): 1325–1331.
- Bymaster, F.P., Falcone, J.F., Bauzon, D., Kennedy, J.S., Schenck, K., DeLapp, N.W. and Cohen, M.L. (2001) Potent antagonism of 5-HT(3) and 5-HT(6) receptors by olanzapine. *Eur. J. Pharmacol.*, 430(2–3): 341–349.
- Canton, H., Verrielle, L. and Colpaert, F.C. (1990) Binding of typical and atypical antipsychotics to 5-HT_{1C} and 5-HT₂ sites: clozapine potently interacts with 5-HT_{1C} sites. *Eur. J. Pharmacol.*, 191(1): 93–96.
- Canton, H., Verrielle, L. and Millan, M.J. (1994) Competitive antagonism of serotonin (5-HT)_{2C} and 5-HT_{2A} receptor-mediated phosphoinositide (PI) turnover by clozapine in the rat: a comparison to other antipsychotics. *Neurosci. Lett.*, 181(1–2): 65–68.
- Carlson, B.B., Wisniecki, A. and Salamone, J.D. (2003) Local injections of the 5-hydroxytryptamine antagonist mianserin into substantia nigra pars reticulata block tremulous jaw movements in rats: studies with a putative model of parkinsonian tremor. *Psychopharmacology (Berl.)*, 165(3): 229–237.
- Carlsson, T., Carta, M., Winkler, C., Bjorklund, A. and Kirik, D. (2007) Serotonin neuron transplants exacerbate L-DOPA-induced dyskinesias in a rat model of Parkinson's disease. *J. Neurosci.*, 27(30): 8011–8022.
- Carta, M., Carlsson, T., Kirik, D. and Bjorklund, A. (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain*, 130(Pt 7): 1819–1833.
- Castro, M.E., Pascual, J., Romon, T., Berciano, J., Figols, J. and Pazos, A. (1998) 5-HT_{1B} receptor binding in degenerative movement disorders. *Brain Res.*, 790(1–2): 323–328.
- Chadha, A., Sur, C., Atack, J. and Duty, S. (2000) The 5HT(1B) receptor agonist, CP-93129, inhibits [(3)H]-GABA release from rat globus pallidus slices and reverses akinesia following intrapallidal injection in the reserpine-treated rat. *Br. J. Pharmacol.*, 130(8): 1927–1932.
- Charles, P.D., Padaliya, B.B., Newman, W.J., Gill, C.E., Covington, C.D., Fang, J.Y., So, S.A., Tramontana, M.G., Konrad, P.E. and Davis, T.L. (2004) Deep brain stimulation of the subthalamic nucleus reduces antiparkinsonian medication costs. *Parkinsonism Relat. Disord.*, 10(8): 475–479.
- Chase, T.N. (1974) Serotonergic-dopaminergic interactions and extrapyramidal function. *Adv. Biochem. Psychopharmacol.*, 11(0): 377–385.
- Chase, T.N. and Ng, L.K. (1972) Central monoamine metabolism in Parkinson's disease. *Arch. Neurol.*, 27(6): 486–491.
- Chen, L., Yung, K.K., Chan, Y.S. and Yung, W.H. (2008) 5-HT excites globus pallidus neurons by multiple receptor mechanisms. *Neuroscience*, 151(2): 439–451.
- Chinaglia, G., Landwehrmeyer, B., Probst, A. and Palacios, J.M. (1993) Serotonergic terminal transporters are differentially affected in Parkinson's disease and progressive

- supranuclear palsy: an autoradiographic study with [3H]citalopram. *Neuroscience*, 54(3): 691–699.
- Chu, Y.X., Liu, J., Feng, J., Wang, Y., Zhang, Q.J. and Li, Q. (2004) [Changes of discharge rate and pattern of 5-hydroxytryptamine neurons of dorsal raphe nucleus in a rat model of Parkinson's disease]. *Sheng Li Xue Bao*, 56(5): 597–602.
- Cicin-Sain, L. and Jenner, P. (1993) Reduction in cortical 5-HT₃ binding sites following a unilateral 6-hydroxydopamine lesion of the medial forebrain bundle in rats. *J. Neurol. Sci.*, 115(1): 105–110.
- Clayton, A.H. (1995) Antidepressant-induced tardive dyskinesia: review and case report. *Psychopharmacol. Bull.*, 31(2): 259–264.
- Collingridge, G.L. and Davies, J. (1981) The influence of striatal stimulation and putative neurotransmitters on identified neurones in the rat substantia nigra. *Brain Res.*, 212(2): 345–359.
- Commins, D.L., Shaughnessy, R.A., Axt, K.J., Vosmer, G. and Seiden, L.S. (1989) Variability among brain regions in the specificity of 6-hydroxydopamine (6-OHDA)-induced lesions. *J. Neural Transm.*, 77(2–3): 197–210.
- Compan, V., Daszuta, A., Salin, P., Sebben, M., Bockaert, J. and Dumuis, A. (1996) Lesion study of the distribution of serotonin 5-HT₄ receptors in rat basal ganglia and hippocampus. *Eur. J. Neurosci.*, 8(12): 2591–2598.
- Compan, V., Segu, L., Buhot, M.C. and Daszuta, A. (1998) Selective increases in serotonin 5-HT_{1B/1D} and 5-HT_{2A/2C} binding sites in adult rat basal ganglia following lesions of serotonergic neurons. *Brain Res.*, 793(1–2): 103–111.
- Cornea-Hébert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, 409(2): 187–209.
- Corvaja, N., Doucet, G. and Bolam, J.P. (1993) Ultrastructure and synaptic targets of the raphe-nigral projection in the rat. *Neuroscience*, 55(2): 417–427.
- D'Addario, C., Di Benedetto, M., Izenwasser, S., Candeletti, S. and Romualdi, P. (2007) Role of serotonin in the regulation of the dynorphinergic system by a kappa-opioid agonist and cocaine treatment in rat CNS. *Neuroscience*, 144(1): 157–164.
- Dahlström, A. and Fuxe, K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.*, 62(232): 1–55.
- Darmani, N.A., Shaddy, J. and Gerdes, C.F. (1996) Differential ontogenesis of three DOI-induced behaviors in mice. *Physiol. Behav.*, 60(6): 1495–1500.
- Davies, J. and Tongroach, P. (1978) Neuropharmacological studies on the nigro-striatal and raphe-striatal system in the rat. *Eur. J. Pharmacol.*, 51(2): 91–100.
- De Deurwaerdere, P. and Chesselet, M.F. (2000) Nigrostriatal lesions alter oral dyskinesia and c-Fos expression induced by the serotonin agonist 1-(m-chlorophenyl)piperazine in adult rats. *J. Neurosci.*, 20(13): 5170–5178.
- del Arco, C., Galende, I. and Pazos, A. (1993) Autoradiographic mapping of 5-HT₁ receptors in the guinea-pig brain with particular reference to the 5-HT_{1D} receptor sites. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 347(3): 248–256.
- DeLong, M.R. (1990) Primate models of movement disorders of basal ganglia origin. *Trends Neurosci.*, 13(7): 281–285.
- Deniau, J.M. and Chevalier, G. (1985) Disinhibition as a basic process in the expression of striatal functions. II. The striato-nigral influence on thalamocortical cells of the ventromedial thalamic nucleus. *Brain Res.*, 334(2): 227–233.
- Di Giovanni, G. (2008) Will it ever become possible to prevent dopaminergic neuronal degeneration? *CNS Neurol. Disord. Drug Targets*, 7(1): 28–44.
- Di Giovanni, G., Di Matteo, V., La Grutta, V. and Esposito, E. (2001) m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience*, 103(1): 111–116.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006a) Central serotonin-2C receptor: from physiology to pathology. *Curr. Top. Med. Chem.*, 6(18): 1909–1925.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Esposito, E. (2006b) Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT_{2C} receptor be a new target for therapeutic strategies? *Curr. Med. Chem.*, 13(25): 3069–3081.
- Dijk, S.N., Francis, P.T., Stratmann, G.C. and Bowen, D.M. (1995) NMDA-induced glutamate and aspartate release from rat cortical pyramidal neurones: evidence for modulation by a 5-HT_{1A} antagonist. *Br. J. Pharmacol.*, 115(7): 1169–1174.
- Di Matteo, V., Cacchio, M., Di Giulio, C., Di Giovanni, G. and Esposito, E. (2002) Biochemical evidence that the atypical antipsychotic drugs clozapine and risperidone block 5-HT_{2C} receptors in vivo. *Pharmacol. Biochem. Behav.*, 71(4): 607–613.
- Doder, M., Rabiner, E.A., Turjanski, N., Lees, A.J. and Brooks, D.J. (2003) Tremor in Parkinson's disease and serotonergic dysfunction: an 11C-WAY 100635 PET study. *Neurology*, 60(4): 601–605.
- Doucet, E., Miquel, M.C., Nosjean, A., Verge, D., Hamon, M. and Emerit, M.B. (1999) Immunolabeling of the rat central nervous system with antibodies partially selective of the short form of the 5-HT₃ receptor. *Neuroscience*, 95(3): 881–892.
- Dray, A., Gonye, T.J. and Oakley, N.R. (1976) Effects of alpha-flupenthixol on dopamine and 5-hydroxytryptamine responses of substantia nigra neurones. *Neuropharmacology*, 15(12): 793–796.
- Duncan, G.E., Knapp, D.J., Breese, G.R., Crews, F.T. and Little, K.Y. (1998) Species differences in regional patterns of 3H-8-OH-DPAT and 3H-zolpidem binding in the rat and human brain. *Pharmacol. Biochem. Behav.*, 60(2): 439–448.
- Durif, F., Debilly, B., Galitzky, M., Morand, D., Viallet, F., Borg, M., Thobois, S., Broussolle, E. and Rascol, O. (2004) Clozapine improves dyskinesias in Parkinson disease: a double-blind, placebo-controlled study. *Neurology*, 62(3): 381–388.

- Duvoisin, R.C. (1967) Cholinergic-anticholinergic antagonism in parkinsonism. *Arch. Neurol.*, 17(2): 124–136.
- Eberle-Wang, K., Lucki, I. and Chesselet, M.F. (1996) A role for the subthalamic nucleus in 5-HT_{2C}-induced oral dyskinesia. *Neuroscience*, 72(1): 117–128.
- Eberle-Wang, K., Mikeladze, Z., Uryu, K. and Chesselet, M.F. (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J. Comp. Neurol.*, 384(2): 233–247.
- Eisensamer, B., Uhr, M., Meyr, S., Gimpl, G., Deiml, T., Rammes, G., Lambert, J.J., Zieglgansberger, W., Holsboer, F. and Rupprecht, R. (2005) Antidepressants and antipsychotic drugs colocalize with 5-HT₃ receptors in raft-like domains. *J. Neurosci.*, 25(44): 10198–10206.
- Ellison, G. (1991) Spontaneous orofacial movements in rodents induced by long-term neuroleptic administration: a second opinion. *Psychopharmacology (Berl.)*, 104(3): 404–408.
- Ellison, G. and See, R.E. (1989) Rats administered chronic neuroleptics develop oral movements which are similar in form to those in humans with tardive dyskinesia. *Psychopharmacology (Berl.)*, 98(4): 564–566.
- el Mansari, M. and Blier, P. (1997) In vivo electrophysiological characterization of 5-HT receptors in the guinea pig head of caudate nucleus and orbitofrontal cortex. *Neuropharmacology*, 36(4–5): 577–588.
- el Mansari, M., Radja, F., Ferron, A., Reader, T.A., Molina-Holgado, E. and Descarries, L. (1994) Hypersensitivity to serotonin and its agonists in serotonin-hyperinnervated neostriatum after neonatal dopamine denervation. *Eur. J. Pharmacol.*, 261(1–2): 171–178.
- Esposito, E., Di Matteo, V., Benigno, A., Pierucci, M., Crescimanno, G. and Di Giovanni, G. (2007a) Non-steroidal anti-inflammatory drugs in Parkinson's disease. *Exp. Neurol.*, 205(2): 295–312.
- Esposito, E., Di Matteo, V. and Di Giovanni, G. (2007b) Death in the substantia nigra: a motor tragedy. *Expert Rev. Neurother.*, 7(6): 677–697.
- Esposito, E., Di Matteo, V., Pierucci, M., Benigno, A. and Di Giovanni, G. (2007c) Role of central 5-HT_{2C} receptor in the control of basal ganglia functions. In: Di Giovanni G. (Ed.), *The basal ganglia pathophysiology: recent advances*. Transworld Research Network, Trivandrum, pp. 97–127.
- Fibiger, H.C. and Miller, J.J. (1977) An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. *Neuroscience*, 2: 975–987.
- Filip, M., Bubar, M.J. and Cunningham, K.A. (2004) Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT₂ receptor subtypes to the hyperlocomotor effects of cocaine: acute and chronic pharmacological analyses. *J. Pharmacol. Exp. Ther.*, 310(3): 1246–1254.
- Filip, M. and Cunningham, K.A. (2002) Serotonin 5-HT_{2C} receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacol. Biochem. Behav.*, 71(4): 745–756.
- Fletcher, P.J., Grottick, A.J. and Higgins, G.A. (2002) Differential effects of the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27(4): 576–586.
- Fletcher, S. and Barnes, N.M. (1999) Autoradiographic localization of the [³H]-(S)-zacopride labelled 5-HT₃ receptor in porcine brain. *Neurosci. Lett.*, 269(2): 91–94.
- Flores, G., Rosales, M.G., Hernandez, S., Sierra, A. and Aceves, J. (1995) 5-Hydroxytryptamine increases spontaneous activity of subthalamic neurons in the rat. *Neurosci. Lett.*, 192(1): 17–20.
- Fox, S.H. and Brotchie, J.M. (2000a) 5-HT_{2C} receptor antagonists enhance the behavioural response to dopamine D(1) receptor agonists in the 6-hydroxydopamine-lesioned rat. *Eur. J. Pharmacol.*, 398(1): 59–64.
- Fox, S.H. and Brotchie, J.M. (2000b) 5-HT_{2C} receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Mov. Disord.*, 15(6): 1064–1069.
- Fox, S.H., Moser, B. and Brotchie, J.M. (1998) Behavioral effects of 5-HT_{2C} receptor antagonism in the substantia nigra zona reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp. Neurol.*, 151(1): 35–49.
- Frechilla, D., Cobreros, A., Saldise, L., Moratalla, R., Insausti, R., Luquin, M. and Del Río, J. (2001) Serotonin 5-HT_{1A} receptor expression is selectively enhanced in the striosomal compartment of chronic parkinsonian monkeys. *Synapse*, 39(4): 288–296.
- Garcia, L., D'Alessandro, G., Bioulac, B. and Hammond, C. (2005) High-frequency stimulation in Parkinson's disease: more or less? *Trends Neurosci.*, 28(4): 209–216.
- Gardell, L.R., Vanover, K.E., Pounds, L., Johnson, R.W., Barido, R., Anderson, G.T., Veinbergs, I., Dyssegaard, A., Brunmark, P., Tabatabaei, A., Davis, R.E., Brann, M.R., Hacksell, U. and Bonhaus, D.W. (2007) ACP-103, a 5-hydroxytryptamine 2A receptor inverse agonist, improves the antipsychotic efficacy and side-effect profile of haloperidol and risperidone in experimental models. *J. Pharmacol. Exp. Ther.*, 322(2): 862–870.
- Gehlert, D.R., Gackenhimer, S.L., Wong, D.T. and Robertson, D.W. (1991) Localization of 5-HT₃ receptors in the rat brain using [³H]LY278584. *Brain Res.*, 553(1): 149–154.
- Gehlert, D.R., Schober, D.A., Gackenhimer, S.L., Mais, D.E., Ladouceur, G. and Robertson, D.W. (1993) Synthesis and evaluation of [¹²⁵I]-(S)-iodozacopride, a high affinity radioligand for 5HT₃ receptors. *Neurochem. Int.*, 23(4): 373–383.
- Gerard, C., el Mestikawy, S., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23(3): 164–173.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 746(1–2): 207–219.
- Gerber, R., Altar, C.A. and Liebman, J.M. (1988) Rotational behavior induced by 8-hydroxy-DPAT, a putative 5HT-1A

- agonist, in 6-hydroxydopamine-lesioned rats. *Psychopharmacology* (Berl.), 94(2): 178–182.
- Gerfen, C.R. (1984) The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems. *Nature*, 311(5985): 461–464.
- Gerfen, C.R. (1985) The neostriatal mosaic. I. Compartmental organization of projections from the striatum to the substantia nigra in the rat. *J. Comp. Neurol.*, 236(4): 454–476.
- Goldstein, M., Anagnoste, B., Battista, A.F., Owen, W.S. and Nakatani, S. (1969) Studies of amines in the striatum in monkeys with nigral lesions. The disposition, biosynthesis and metabolites of [³H]dopamine and [¹⁴C]serotonin in the striatum. *J. Neurochem.*, 16(4): 645–653.
- Gong, L. and Kostrzewa, R.M. (1992) Supersensitized oral responses to a serotonin agonist in neonatal 6-OHDA-treated rats. *Pharmacol. Biochem. Behav.*, 41(3): 621–623.
- Gongora-Alfaro, J.L., Hernandez-Lopez, S., Flores-Hernandez, J. and Galarraga, E. (1997) Firing frequency modulation of substantia nigra reticulata neurons by 5-hydroxytryptamine. *Neurosci. Res.*, 29(3): 225–231.
- Granoff, M.I. and Ashby, C.R., Jr. (1998) The effect of the repeated administration of the compound 3,4-methylenedioxymethamphetamine on the response of rats to the 5-HT_{2A/C} receptor agonist (+/–)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). *Neuropsychobiology*, 37(1): 36–40.
- Grottick, A.J., Fletcher, P.J. and Higgins, G.A. (2000) Studies to investigate the role of 5-HT_{2C} receptors on cocaine- and food-maintained behavior. *J. Pharmacol. Exp. Ther.*, 295(3): 1183–1191.
- Guerra, M.J., Liste, I. and Labandeira-Garcia, J.L. (1997) Effects of lesions of the nigrostriatal pathway and of nigral grafts on striatal serotonergic innervation in adult rats. *Neuroreport*, 8(16): 3485–3488.
- Guiard, B.P., el Mansari, M., Merali, Z. and Blier, P. (2008) Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *Int. J. Neuropsychopharmacol.*, 21: 1–15.
- Gunes, A., Dahl, M.-L., Spina, E. and Scordo, M. (2008) Further evidence for the association between 5-HT_{2C} receptor gene polymorphisms and extrapyramidal side effects in male schizophrenic patients. *Eur. J. Clin. Pharmacol.*, 64(5): 477–482.
- Gunes, A., Scordo, M., Jaanson, P. and Dahl, M.-L. (2007) Serotonin and dopamine receptor gene polymorphisms and the risk of extrapyramidal side effects in perphenazine-treated schizophrenic patients. *Psychopharmacology* (Berl.), 190(4): 479–484.
- Haapaniemi, T.H., Ahonen, A., Torniaainen, P., Sotaniemi, K.A. and Myllyla, V.V. (2001) [¹²³I]beta-CIT SPECT demonstrates decreased brain dopamine and serotonin transporter levels in untreated parkinsonian patients. *Mov. Disord.*, 16(1): 124–130.
- Haleem, D.J. and Khan, N.H. (2003) Enhancement of serotonin-1A receptor dependent responses following withdrawal of haloperidol in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27(4): 645–651.
- Haleem, D.J., Samad, N. and Haleem, M.A. (2007a) Reversal of haloperidol-induced extrapyramidal symptoms by buspirone: a time-related study. *Behav. Pharmacol.*, 18(2): 147–153.
- Haleem, D.J., Samad, N. and Haleem, M.A. (2007b) Reversal of haloperidol-induced tardive vacuuous chewing movements and supersensitive somatodendritic serotonergic response by buspirone in rats. *Pharmacol. Biochem. Behav.*, 87(1): 115–121.
- Haleem, D.J., Shireen, E. and Haleem, M.A. (2004) Somatodendritic and postsynaptic serotonin-1A receptors in the attenuation of haloperidol-induced catalepsy. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 28(8): 1323–1329.
- Hall, H., Lundkvist, C., Halldin, C., Farde, L., Pike, V.W., McCarron, J.A., Fletcher, A., Cliffe, I.A., Barf, T., Wikstrom, H. and Sedvall, G. (1997) Autoradiographic localization of 5-HT_{1A} receptors in the post-mortem human brain using [³H]WAY-100635 and [¹¹C]WAY-100635. *Brain Res.*, 745(1–2): 96–108.
- Halliday, G.M., Blumbergs, P.C., Cotton, R.G.H., Blessing, W.W. and Geffen, L.B. (1990) Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. *Brain Res.*, 510(1): 104–107.
- Hameleers, R., Blokland, A., Steinbusch, H.W.M., Visser-Vandewalle, V. and Temel, Y. (2007) Hypomobility after DOI administration can be reversed by subthalamic nucleus deep brain stimulation. *Behav. Brain Res.*, 185(1): 65–67.
- Hamon, M., Doucet, E., Lefevre, K., Miquel, M.C., Lanfumey, L., Insausti, R., Frechilla, D., Del Rio, J. and Verge, D. (1999) Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT₆ receptors. *Neuropsychopharmacology*, 21(2 Suppl): 68S–76S.
- Hashimoto, K. and Kita, H. (2008) Serotonin activates presynaptic and postsynaptic receptors in rat globus pallidus. *J. Neurophysiol.*, 99(4): 1723–1732.
- Hawkins, M.F., Uzelac, S.M., Baumeister, A.A., Hearn, J.K., Broussard, J.I. and Guillot, T.S. (2002) Behavioral responses to stress following central and peripheral injection of the 5-HT₂ agonist DOI. *Pharmacol. Biochem. Behav.*, 73(3): 537–544.
- Herrick-Davis, K., Grinde, E. and Teitler, M. (2000) Inverse agonist activity of atypical antipsychotic drugs at human 5-hydroxytryptamine_{2C} receptors. *J. Pharmacol. Exp. Ther.*, 295(1): 226–232.
- Hervé, D., Pickel, V.M., Joh, T.H. and Beaudet, A. (1987) Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. *Brain Res.*, 435(1–2): 71–83.
- Hicks, P.B. (1990) The effect of serotonergic agents on haloperidol-induced catalepsy. *Life Sci.*, 47(18): 1609–1615.
- Higgins, G.A., Jordan, C.C. and Skingle, M. (1991) Evidence that the unilateral activation of 5-HT_{1D} receptors in the substantia nigra of the guinea-pig elicits contralateral rotation. *Br. J. Pharmacol.*, 102(2): 305–310.
- Hirst, W.D., Abrahamsen, B., Blaney, F.E., Calver, A.R., Aloj, L., Price, G.W. and Medhurst, A.D. (2003) Differences in the central nervous system distribution and pharmacology of the

- mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. *Mol. Pharmacol.*, 64: 1295–1308.
- Hirst, W.D., Minton, J.A., Bromidge, S.M., Moss, S.F., Latter, A.J., Riley, G., Routledge, C., Middlemiss, D.N. and Price, G.W. (2000) Characterization of [(125)I]-SB-258585 binding to human recombinant and native 5-HT₆ receptors in rat, pig and human brain tissue. *Br. J. Pharmacol.*, 130(7): 1597–1605.
- Hoffman, B.J. and Mezey, E. (1989) Distribution of serotonin 5-HT_{1C} receptor mRNA in adult rat brain. *FEBS Lett.*, 247(2): 453–462.
- Horner, K.A., Adams, D.H., Hanson, G.R. and Keefe, K.A. (2005) Blockade of stimulant-induced preprodynorphin mRNA expression in the striatal matrix by serotonin depletion. *Neuroscience*, 131(1): 67–77.
- Hornykiewicz, O. (1973) Dopamine in the basal ganglia: its role and therapeutic implications (including the clinical use of L-DOPA). *Br. Med. Bull.*, 29(2): 172–178.
- Hornykiewicz, O. (1998) Biochemical aspects of Parkinson's disease. *Neurology*, 51(2): S2–S9.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71(4): 533–554.
- Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M. (1986) Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. *Brain Res.*, 376(1): 85–96.
- Ikeguchi, K. and Kuroda, A. (1995) Mianserin treatment of patients with psychosis induced by antiparkinsonian drugs. *Eur. Arch. Psychiatry Clin. Neurosci.*, 244(6): 320–324.
- Ikram, H., Samad, N. and Haleem, D.J. (2007) Neurochemical and behavioral effects of m-CPP in a rat model of tardive dyskinesia. *Pak. J. Pharm. Sci.*, 20(3): 188–195.
- Invernizzi, R.W., Cervo, L. and Samanin, R. (1988) 8-Hydroxy-2-(di-n-propylamino) tetralin, a selective serotonin_{1A} receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. *Neuropharmacology*, 27(5): 515–518.
- Invernizzi, R.W., Pierucci, M., Calcagno, E., Di Giovanni, G., Di Matteo, V., Benigno, A. and Esposito, E. (2007) Selective activation of 5-HT_{2C} receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience*, 144(4): 1523–1535.
- Ito, H., Halldin, C. and Farde, L. (1999) Localization of 5-HT_{1A} receptors in the living human brain using [carbonyl-¹¹C]WAY-100635: PET with anatomic standardization technique. *J. Nucl. Med.*, 40(1): 102–109.
- Jackson, M.J., Al-Barghouthy, G., Pearce, R.K.B., Smith, L., Hagan, J.J. and Jenner, P. (2004) Effect of 5-HT_{1B/D} receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. *Pharmacol. Biochem. Behav.*, 79(3): 391–400.
- Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.*, 72(1): 165–229.
- James, T.A. and Starr, M.S. (1980) Rotational behaviour elicited by 5-HT in the rat: evidence for an inhibitory role of 5-HT in the substantia nigra and corpus striatum. *J. Pharm. Pharmacol.*, 32(3): 196–200.
- Jellinger, K. (1987) Overview of morphological changes in Parkinson's disease. *Adv. Neurol.*, 45: 1–18.
- Johnson, S.W., Mercuri, N.B. and North, R.A. (1992) 5-hydroxytryptamine_{1B} receptors block the GABA_B synaptic potential in rat dopamine neurons. *J. Neurosci.*, 12(5): 2000–2006.
- Johnston, T.H. and Brotchie, J.M. (2006) Drugs in development for Parkinson's disease: an update. *Curr. Opin. Investig. Drugs*, 7(1): 25–32.
- Kalkman, H.O., Neumann, V. and Tricklebank, M.D. (1997) Clozapine inhibits catalepsy induced by olanzapine and loxapine, but prolongs catalepsy induced by SCH 23390 in rats. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 355(3): 361–364.
- Kehne, J.H., Ketteler, H.J., McCloskey, T.C., Sullivan, C.K., Dudley, M.W. and Schmidt, C.J. (1996) Effects of the selective 5-HT_{2A} receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. *Neuropsychopharmacology*, 15(2): 116–124.
- Kennett, G.A., Lightowler, S., de Biasi, V., Stevens, N.C., Wood, M.D., Tulloch, I.F. and Blackburn, T.P. (1994) Effect of chronic administration of selective 5-hydroxytryptamine and noradrenaline uptake inhibitors on a putative index of 5-HT_{2C/2B} receptor function. *Neuropharmacology*, 33(12): 1581–1588.
- Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P. and Blackburn, T.P. (1996) In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C/5-HT_{2B}} receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.*, 117(3): 427–434.
- Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, V., Avenell, K.Y., Stean, T., Upton, N., Bromidge, S., Forbes, I.T., Brown, A.M., Middlemiss, D.N. and Blackburn, T.P. (1997) SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacology*, 36(4–5): 609–620.
- Kerenyi, L., Ricaurte, G.A., Schretlen, D.J., McCann, U., Varga, J., Mathews, W.B., Ravert, H.T., Dannals, R.F., Hilton, J., Wong, D.F. and Szabo, Z. (2003) Positron emission tomography of striatal serotonin transporters in Parkinson disease. *Arch. Neurol.*, 60(9): 1223–1229.
- Khawaja, X. (1995) Quantitative autoradiographic characterisation of the binding of [³H]WAY-100635, a selective 5-HT_{1A} receptor antagonist. *Brain Res.*, 673(2): 217–225.
- Kienzl, E., Riederer, P., Jellinger, K. and Wesemann, W. (1981) Transitional states of central serotonin receptors in Parkinson's disease. *J. Neural. Transm.*, 51(1–2): 113–122.
- Kim, S.E., Choi, J.Y., Choe, Y.S., Choi, Y. and Lee, W.Y. (2003) Serotonin transporters in the midbrain of Parkinson's

- disease patients: a study with ^{123}I -{beta}-CIT SPECT. *J. Nucl. Med.*, 44(6): 870–876.
- Kish, S.J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L.-J., Guttman, M. and Furukawa, Y. (2008) Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. *Brain*, 131(1): 120–131.
- Kita, H., Chiken, S., Tachibana, Y. and Nambu, A. (2007) Serotonin modulates pallidal neuronal activity in the awake monkey. *J. Neurosci.*, 27(1): 75–83.
- Kita, H. and Kitai, S.T. (1987) Efferent projections of the subthalamic nucleus in the rat: light and electron microscopic analysis with the PHA-L method. *J. Comp. Neurol.*, 260(3): 435–452.
- Kleven, M.S., Barret-Grevoz, C., Slot, L.B. and Newman-Tancredi, A. (2005) Novel antipsychotic agents with 5-HT_{1A} agonist properties: Role of 5-HT_{1A} receptor activation in attenuation of catalepsy induction in rats. *Neuropharmacology*, 49(2): 135–143.
- Knobelman, D.A., Kung, H.F. and Lucki, I. (2000) Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT_{1A} and 5-HT_{1B} receptors. *J. Pharmacol. Exp. Ther.*, 292(3): 1111–1117.
- Kohen, R., Metcalf, M.A., Khan, N., Druck, T., Huebner, K., Lachowicz, J.E., Meltzer, H.Y., Sibley, D.R., Roth, B.L. and Hamblin, M.W. (1996) Cloning, characterization, and chromosomal localization of a human 5-HT₆ serotonin receptor. *J. Neurochem.*, 66(1): 47–56.
- Krack, P., Batir, A., Van Blercom, N., Chabardes, S., Fraix, V., Ardouin, C., Koudsie, A., Limousin, P.D., Benazzouz, A., LeBas, J.F., Benabid, A.-L. and Pollak, P. (2003) Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson's disease. *N. Engl. J. Med.*, 349(20): 1925–1934.
- Krack, P., Benazzouz, A., Pollak, P., Limousin, P., Piallat, B., Hoffmann, D., Xie, J. and Benabid, A.L. (1998) Treatment of tremor in Parkinson's disease by subthalamic nucleus stimulation. *Mov. Disord.*, 13(6): 907–914.
- Krebs-Thomson, K. and Geyer, M.A. (1996) The role of 5-HT_{1A} receptors in the locomotor-suppressant effects of LSD: WAY-100635 studies of 8-OH-DPAT, DOI and LSD in rats. *Behav. Pharmacol.*, 7(6): 551–559.
- Kung, M.P., Frederick, D., Mu, M., Zhuang, Z.P. and Kung, H.F. (1995) 4-(2'-Methoxy-phenyl)-1-[2'-(n-2"-pyridinyl)-p-iodobenzamido]-ethyl-piperazine ([125I]p-MPPI) as a new selective radioligand of serotonin-1A sites in rat brain: in vitro binding and autoradiographic studies. *J. Pharmacol. Exp. Ther.*, 272(1): 429–437.
- Kuoppamaki, M., Palvimaki, E.P., Hietala, J. and Syvalahti, E. (1995) Differential regulation of rat 5-HT_{2A} and 5-HT_{2C} receptors after chronic treatment with clozapine, chlorpromazine and three putative atypical antipsychotic drugs. *Neuropsychopharmacology*, 13(2): 139–150.
- Kuoppamaki, M., Syvalahti, E. and Hietala, J. (1993) Clozapine and N-desmethylclozapine are potent 5-HT_{1C} receptor antagonists. *Eur. J. Pharmacol.*, 245(2): 179–182.
- Laporte, A.M., Koscielniak, T.M., Ponchant, D., Vergé, M., Hamon, H. and Gozlan, H. (1992) Quantitative autoradiographic mapping of 5-HT₃ receptors in the rat CNS using [^{125}I]iodo-zacopride and [^3H]zacopride as radioligands. *Synapse*, 10(4): 271–281.
- Laprade, N., Radja, F., Reader, T.A. and Soghomonian, J.-J. (1996) Dopamine receptor agonists regulate levels of the serotonin 5-HT_{2A} receptor and its mRNA in a subpopulation of rat striatal neurons. *J. Neurosci.*, 16(11): 3727–3736.
- Lavoie, B. and Parent, A. (1990) Immunohistochemical study of the serotonergic innervation of the basal ganglia in the squirrel monkey. *J. Comp. Neurol.*, 299(1): 1–16.
- Lee, M.S., Rinne, J.O. and Marsden, C.D. (2000) The pedunculopontine nucleus: its role in the genesis of movement disorders. *Yonsei Med. J.*, 41(2): 167–184.
- Leentjens, A.F.G. (2004) Depression in Parkinson's disease: conceptual issues and clinical challenges. *J. Geriatr. Psychiatr. Neurol.*, 17(3): 120–126.
- Leentjens, A.F.G., Scholtissen, B., Vreeling, F.W. and Verhey, F.R.J. (2006) The serotonergic hypothesis for depression in Parkinson's disease: an experimental approach. *Neuropsychopharmacology*, 31(5): 1009–1015.
- Leysen, J.E., Gommeren, W., Van Gompel, P., Wynants, J., Janssen, P.F. and Laduron, P.M. (1985) Receptor-binding properties in vitro and in vivo of ritanserin: a very potent and long acting serotonin-5₂ antagonist. *Mol. Pharmacol.*, 27(6): 600–611.
- Limousin, P., Pollak, P., Benazzouz, A., Hoffmann, D., Le Bas, J.F., Broussolle, E., Perret, J.E. and Benabid, A.L. (1995) Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *Lancet*, 345(8942): 91–95.
- Lopez-Gimenez, J.F., Mengod, G., Palacios, J.M. and Vilaro, M.T. (1997) Selective visualization of rat brain 5-HT_{2A} receptors by autoradiography with [^3H]MDL 100,907. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 356(4): 446–454.
- Lopez-Gimenez, J.F., Mengod, G., Palacios, J.M. and Vilaro, M.T. (1999) Human striosomes are enriched in 5-HT_{2A} receptors: autoradiographical visualization with [^3H]MDL100,907, [^{125}I](±)DOI and [^3H]ketanserin. *Eur. J. Neurosci.*, 11(10): 3761–3765.
- Lucas, G., Bonhomme, N., Deurwaerdère, P.D., Moal, M.L. and Spampinato, U. (1997) 8-OH-DPAT, a 5-HT_{1A} agonist and ritanserin, a 5-HT_{2A/C} antagonist, reverse haloperidol-induced catalepsy in rats independently of striatal dopamine release. *Psychopharmacology (Berl.)*, 131(1): 57–63.
- Lucas, G., De Deurwaerdère, P., Caccia, S. and Umberto, S. (2000) The effect of serotonergic agents on haloperidol-induced striatal dopamine release in vivo: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology*, 39(6): 1053–1063.
- Maeda, T., Kannari, K., Shen, H., Arai, A., Tomiyama, M., Matsunaga, M. and Suda, T. (2003) Rapid induction of serotonergic hyperinnervation in the adult rat striatum with extensive dopaminergic denervation. *Neurosci. Lett.*, 343(1): 17–20.
- Mailly, P., Charpier, S., Menetrey, A. and Deniau, J.-M. (2003) Three-dimensional organization of the recurrent axon

- collateral network of the substantia nigra pars reticulata neurons in the rat. *J. Neurosci.*, 23: 5247–5257.
- Mander, A., McCausland, M., Workman, B., Flamer, H. and Christophidis, N. (1994) Fluoxetine induced dyskinesia. *Aust. N. Z. J. Psychiatry*, 28(2): 328–330.
- Marazziti, D., Betti, L., Giannaccini, G., Rossi, A., Masala, I., Baroni, S., Cassano, G. and Lucacchini, A. (2001) Distribution of [3H]GR65630 binding in human brain postmortem. *Neurochem. Res.*, 26(3): 187–190.
- Martin-Cora, F.J. and Pazos, A. (2004) Autoradiographic distribution of 5-HT₇ receptors in the human brain using [3H]mesulergine: comparison to other mammalian species. *Br. J. Pharmacol.*, 141(1): 92–104.
- Martinez-Price, D.L. and Geyer, M.A. (2002) Subthalamic 5-HT_{1A} and 5-HT_{1B} receptor modulation of RU 24969-induced behavioral profile in rats. *Pharmacol. Biochem. Behav.*, 71(4): 569–580.
- Matsubara, K., Shimizu, K., Suno, M., Ogawa, K., Awaya, T., Yamada, T., Noda, T., Satomi, M., Ohtaki, K.-I., Chiba, K., Tasaki, Y. and Shiono, H. (2006) Tansospirone, a 5-HT_{1A} agonist, ameliorates movement disorder via non-dopaminergic systems in rats with unilateral 6-hydroxydopamine-generated lesions. *Brain Res.*, 1112(1): 126–133.
- Mayeux, R. (1990) The “serotonin hypothesis” for depression in Parkinson’s disease. *Adv. Neurol.*, 53: 163–166.
- McQuade, R. and Sharp, T. (1995) Release of cerebral 5-hydroxytryptamine evoked by electrical stimulation of the dorsal and median raphe nuclei: effect of a neurotoxic amphetamine. *Neuroscience*, 68(4): 1079–1088.
- Mehta, A., Eberle-Wang, K. and Chesselet, M.-F. (2001) Increased m-CPP-induced oral dyskinesia after lesion of serotonergic neurons. *Pharmacol. Biochem. Behav.*, 68(2): 347–353.
- Mendlin, A., Martin, F.J. and Jacobs, B.L. (1999) Dopaminergic input is required for increases in serotonin output produced by behavioral activation: an in vivo microdialysis study in rat forebrain. *Neuroscience*, 93(3): 897–905.
- Mengod, G., Pompeiano, M., Martinez-Mir, M.I. and Palacios, J.M. (1990) Localization of the mRNA for the 5-HT₂ receptor by in situ hybridization histochemistry. Correlation with the distribution of receptor sites. *Brain Res.*, 524(1): 139–143.
- Mengod, G., Vilaro, M.T., Raurich, A., Lopez-Gimenez, J.F., Cortes, R. and Palacios, J.M. (1996) 5-HT receptors in mammalian brain: receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. *Histochem. J.*, 28(11): 747–758.
- Middlemiss, D.N. and Hutson, P.H. (1990) The 5-HT_{1B} receptors. *Ann. N. Y. Acad. Sci.*, 600: 132–147. discussion 347–348.
- Mignon, L. and Wolf, W. (2002) Postsynaptic 5-HT_{1A} receptors mediate an increase in locomotor activity in the monoamine-depleted rat. *Psychopharmacology (Berl.)*, 163(1): 85–94.
- Mignon, L. and Wolf, W. (2007) Postsynaptic 5-HT_{1A} receptor stimulation increases motor activity in the 6-hydroxydopamine-lesioned rat: implications for treating Parkinson’s disease. *Psychopharmacology (Berl.)*, 192(1): 49–59.
- Mignon, L.J. and Wolf, W.A. (2005) 8-Hydroxy-2-(di-n-propylamino)tetralin reduces striatal glutamate in an animal model of Parkinson’s disease. *Neuroreport*, 16(7): 699–703.
- Miller, C.H., Fleischhacker, W.W., Ehrmann, H. and Kane, J.M. (1990) Treatment of neuroleptic induced akathisia with the 5-HT₂ antagonist ritanserin. *Psychopharmacol. Bull.*, 26(3): 373–376.
- Miller, K.M., Okun, M.S., Fernandez, H.F., Jacobson, C.E., Rodriguez, R.L. and Bowers, D. (2007) Depression symptoms in movement disorders: comparing Parkinson’s disease, dystonia, and essential tremor. *Mov. Disord.*, 22(5): 666–672.
- Miquel, M.C., Doucet, E., Boni, C., El Mestikawy, S., Matthiessen, L., Daval, G., Verge, D. and Hamon, M. (1991) Central serotonin_{1A} receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridization histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. *Neurochem. Int.*, 19(4): 453–465.
- Mitchell, I.J., Sambrook, M.A. and Crossman, A.R. (1985) Subcortical changes in the regional uptake of [3H]-2-deoxyglucose in the brain of the monkey during experimental choreiform dyskinesia elicited by injection of a gamma-aminobutyric acid antagonist into the subthalamic nucleus. *Brain*, 108(2): 405–422.
- Miyawaki, E., Meah, Y. and Koller, W.C. (1997) Serotonin, dopamine, and motor effects in Parkinson’s disease. *Clin. Neuropharmacol.*, 20(4): 300–310.
- Molina-Holgado, E., Dewar, K.M., Descarries, L. and Reader, T.A. (1994) Altered dopamine and serotonin metabolism in the dopamine-denervated and serotonin-hyperinnervated neostriatum of adult rat after neonatal 6-hydroxydopamine. *J. Pharmacol. Exp. Ther.*, 270(2): 713–721.
- Monsma, F.J., Jr., Shen, Y., Ward, R.P., Hamblin, M.W. and Sibley, D.R. (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.*, 43(3): 320–327.
- Morales, M., Battenberg, E. and Bloom, F.E. (1998) Distribution of neurons expressing immunoreactivity for the 5HT₃ receptor subtype in the rat brain and spinal cord. *J. Comp. Neurol.*, 402(3): 385–401.
- Moukhes, H., Bosler, O., Bolam, J.P., Vallée, A., Umbriaco, D., Geffard, M. and Doucet, G. (1997) Quantitative and morphometric data indicate precise cellular interactions between serotonin terminals and postsynaptic targets in rat substantia nigra. *Neuroscience*, 76(4): 1159–1171.
- Mrini, A., Soucy, J.-P., Lafaille, F., Lemoine, P. and Descarries, L. (1995) Quantification of the serotonin hyperinnervation in adult rat neostriatum after neonatal 6-hydroxydopamine lesion of nigral dopamine neurons. *Brain Res.*, 669(2): 303–308.
- Naidu, P.S. and Kulkarni, S.K. (2001a) Effect of 5-HT_{1A} and 5-HT_{2A/2C} receptor modulation on neuroleptic-induced vacuous chewing movements. *Eur. J. Pharmacol.*, 428(1): 81–86.

- Naidu, P.S. and Kulkarni, S.K. (2001b) Reversal of neuroleptic-induced orofacial dyskinesia by 5-HT₃ receptor antagonists. *Eur. J. Pharmacol.*, 420(2–3): 113–117.
- Navailles, S., De Deurwaerdère, P. and Spampinato, U. (2006) Clozapine and haloperidol differentially alter the constitutive activity of central serotonin_{2C} receptors in vivo. *Biol. Psychiatry*, 59(6): 568–575.
- Nayak, S.V., Ronde, P., Spier, A.D., Lummis, S.C.R. and Nichols, R.A. (1999) Calcium changes induced by presynaptic 5-hydroxytryptamine-3 serotonin receptors on isolated terminals from various regions of the rat brain. *Neuroscience*, 91(1): 107–117.
- Neal-Beliveau, B.S., Joyce, J.N. and Lucki, I. (1993) Serotonergic involvement in haloperidol-induced catalepsy. *J. Pharmacol. Exp. Ther.*, 265(1): 207–217.
- Nichols, R.A. and Mollard, P. (1996) Direct observation of serotonin 5-HT₃ receptor-induced increases in calcium levels in individual brain nerve terminals. *J. Neurochem.*, 67(2): 581–592.
- Nicholson, S.L. and Brotchie, J.M. (2002) 5-Hydroxytryptamine (5-HT, serotonin) and Parkinson's disease-opportunities for novel therapeutics to reduce the problems of levodopa therapy. *Eur. J. Neurol.*, 9(Suppl 3): 1–6.
- Nishio, H., Kohno, Y., Fujii, A., Negishi, Y., Inoue, A. and Nakata, Y. (1996) 5-HT₃ receptor blocking properties of the antiparkinsonian agent, talipexole. *Gen. Pharmacol.*, 27(5): 779–785.
- Numan, S., Lundgren, K.H., Wright, D.E., Herman, J.P. and Seroogy, K.B. (1995) Increased expression of 5HT₂ receptor mRNA in rat striatum following 6-OHDA lesions of the adult nigrostriatal pathway. *Brain Res. Mol. Brain Res.*, 29(2): 391–396.
- O'Neill, M.F., Heron-Maxwell, C.L. and Shaw, G. (1999) 5-HT₂ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. *Pharmacol. Biochem. Behav.*, 63(2): 237–243.
- Oberlander, C., Demassey, Y., Verdu, A., Van de Velde, D. and Bardelay, C. (1987) Tolerance to the serotonin 5-HT₁ agonist RU 24969 and effects on dopaminergic behaviour. *Eur. J. Pharmacol.*, 139(2): 205–214.
- Oberlander, C., Hunt, P.F., Dumont, C. and Boissier, J.R. (1981) Dopamine independent rotational response to unilateral intranigral injection of serotonin. *Life Sci.*, 28(23): 2595–2601.
- Okazawa, H., Yamane, F., Blier, P. and Diksic, M. (1999) Effects of acute and chronic administration of the serotonin_{1A} agonist buspirone on serotonin synthesis in the rat brain. *J. Neurochem.*, 72(5): 2022–2031.
- Oliver, K.R., Kinsey, A.M., Wainwright, A. and Sirinathsinghji, D.J. (2000) Localization of 5-HT_{5A} receptor-like immunoreactivity in the rat brain. *Brain Res.*, 867(1–2): 131–142.
- Olpe, H.R. and Koella, W.P. (1977) The response of striatal cells upon stimulation of the dorsal and median raphe nuclei. *Brain Res.*, 122(2): 357–360.
- Pakhotin, P. and Bracci, E. (2007) Cholinergic interneurons control the excitatory input to the striatum. *J. Neurosci.*, 27(2): 391–400.
- Park, M.R., Gonzales-Vegas, J.A. and Kitai, S.T. (1982) Serotonergic excitation from dorsal raphe stimulation recorded intracellularly from rat caudate-putamen. *Brain Res.*, 243(1): 49–58.
- Parker, R.M.C., Barnes, J.M., Ge, J., Barber, P.C. and Barnes, N.M. (1996) Autoradiographic distribution of [³H]-(S)-zacopride-labelled 5-HT₃ receptors in human brain. *J. Neurol. Sci.*, 144(1–2): 119–127.
- Pasqualetti, M., Nardi, I., Ladinsky, H., Marazziti, D. and Cassano, G.B. (1996) Comparative anatomical distribution of serotonin 1A, 1D[α] and 2A receptor mRNAs in human brain postmortem. *Brain Res. Mol. Brain Res.*, 39(1–2): 223–233.
- Paulus, W. and Jellinger, K. (1991) The neuropathologic basis of different clinical subgroups of Parkinson's disease. *J. Neuropathol. Exp. Neurol.*, 50(6): 743–755.
- Pazos, A., Cortes, R. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res.*, 346(2): 231–249.
- Pazos, A. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, 346(2): 205–230.
- Pazos, A., Probst, A. and Palacios, J.M. (1987a) Serotonin receptors in the human brain. III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience*, 21(1): 97–122.
- Pazos, A., Probst, A. and Palacios, J.M. (1987b) Serotonin receptors in the human brain. IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*, 21(1): 123–139.
- Perkins, M.N. and Stone, T.W. (1983) Neuronal responses to 5-hydroxytryptamine and dorsal raphe stimulation within the globus pallidus of the rat. *Exp. Neurol.*, 79(1): 118–129.
- Phelps, P.E., Houser, C.R. and Vaughn, J.E. (1985) Immunocytochemical localization of choline acetyltransferase within the rat neostriatum: a correlated light and electron microscopic study of cholinergic neurons and synapses. *J. Comp. Neurol.*, 238(3): 286–307.
- Pike, V.W., McCarron, J.A., Lammerstma, A.A., Hume, S.P., Poole, K., Grasby, P.M., Malizia, A., Cliffe, I.A., Fletcher, A. and Bench, C.J. (1995) First delineation of 5-HT_{1A} receptors in human brain with PET and [¹¹C]WAY-100635. *Eur. J. Pharmacol.*, 283(1–3): R1–R3.
- Pineyro, G. and Blier, P. (1999) Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol. Rev.*, 51(3): 533–591.
- Pires, J.G., Bonikovski, V. and Futuro-Neto, H.A. (2005) Acute effects of selective serotonin reuptake inhibitors on neuroleptic-induced catalepsy in mice. *Braz. J. Med. Biol. Res.*, 38(12): 1867–1872.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12(2): 440–453.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Brain Res. Mol. Brain Res.*, 23(1–2): 163–178.

- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E. and Spampinato, U. (2002) 5-HT_{2A} and 5-HT_{2C/2B} receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26(3): 311–324.
- Prinssen, E.P., Koek, W. and Kleven, M.S. (2000) The effects of antipsychotics with 5-HT(2C) receptor affinity in behavioral assays selective for 5-HT(2C) receptor antagonist properties of compounds. *Eur. J. Pharmacol.*, 388(1): 57–67.
- Queiroz, C.M. and Frussa-Filho, R. (1999) Effects of buspirone on an animal model of tardive dyskinesia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 23(8): 1405–1418.
- Querejeta, E., Oviedo-Chavez, A., Araujo-Alvarez, J.M., Quinones-Cardenas, A.R. and Delgado, A. (2005) In vivo effects of local activation and blockade of 5-HT_{1B} receptors on globus pallidus neuronal spiking. *Brain Res.*, 1043(1–2): 186–194.
- Quirion, R. and Richard, J. (1987) Differential effects of selective lesions of cholinergic and dopaminergic neurons on serotonin-type 1 receptors in rat brain. *Synapse*, 1(1): 124–130.
- Radja, F., Descarries, L., Dewar, K.M. and Reader, T.A. (1993) Serotonin 5-HT₁ and 5-HT₂ receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. *Brain Res.*, 606(2): 273–285.
- Rammes, G., Eisensamer, B., Ferrari, U., Shapa, M., Gimpl, G., Gilling, K., Parsons, C., Riering, K., Hapfelmeier, G., Bondy, B., Zieglansberger, W., Holsboer, F. and Rupprecht, R. (2004) Antipsychotic drugs antagonize human serotonin type 3 receptor currents in a noncompetitive manner. *Mol. Psychiatry*, 9(9): 846–858.
- Rausser, L., Savage, J.E., Meltzer, H.Y. and Roth, B.L. (2001) Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. *J. Pharmacol. Exp. Ther.*, 299(1): 83–89.
- Reavill, C., Kettle, A., Holland, V., Riley, G. and Blackburn, T.P. (1999) Attenuation of haloperidol-induced catalepsy by a 5-HT_{2C} receptor antagonist. *Br. J. Pharmacol.*, 126(3): 572–574.
- Rees, S., den Daas, I., Foord, S., Goodson, S., Bull, D., Kilpatrick, G. and Lee, M. (1994) Cloning and characterization of the human 5-HT_{5A} serotonin receptor. *FEBS Lett.*, 355(3): 242–246.
- Rempel, N.L., Callaway, C.W. and Geyer, M.A. (1993) Serotonin_{1B} receptor activation mimics behavioral effects of presynaptic serotonin release. *Neuropsychopharmacology*, 8(3): 201–211.
- Reynolds, G.P. (2004) Receptor mechanisms in the treatment of schizophrenia. *J. Psychopharmacol. (Oxford)*, 18(3): 340–345.
- Reynolds, G.P., Mason, S.L., Meldrum, A., De Keczer, S., Parnes, H., Eglon, R.M. and Wong, E.H. (1995) 5-Hydroxytryptamine (5-HT)₄ receptors in post mortem human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases. *Br. J. Pharmacol.*, 114(5): 993–998.
- Riad, M., Mestikawy, S.E., Verge, D., Gozlan, H. and Hamon, M. (1991) Visualization and quantification of central 5-HT_{1A} receptors with specific antibodies. *Neurochem. Int.*, 19(4): 413–423.
- Rick, C.E., Stanford, I.M. and Lacey, M.G. (1995) Excitation of rat substantia nigra pars reticulata neurons by 5-hydroxytryptamine in vitro: evidence for a direct action mediated by 5-hydroxytryptamine_{2C} receptors. *Neuroscience*, 69(3): 903–913.
- Ring, H.A. and Serra-Mestre, J. (2002) Neuropsychiatry of the basal ganglia. *J. Neurol. Neurosurg. Psychiatry*, 72: 12–21.
- Roberts, J.C., Reavill, C., East, S.Z., Harrison, P.J., Patel, S., Routledge, C. and Leslie, R.A. (2002) The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT(6) receptor antagonist [(125)I]SB-258585. *Brain Res.*, 934(1): 49–57.
- Rodríguez, J.J., Garcia, D.R. and Pickel, V.M. (1999) Subcellular distribution of 5-hydroxytryptamine_{2A} and *N*-methyl-D-aspartate receptors within single neurons in rat motor and limbic striatum. *J. Comp. Neurol.*, 413(2): 219–231.
- Ronde, P. and Nichols, R.A. (1998) High calcium permeability of serotonin 5-HT₃ receptors on presynaptic nerve terminals from rat striatum. *J. Neurochem.*, 70(3): 1094–1103.
- Rosengarten, H., Bartoszyk, G.D., Quartermain, D. and Lin, Y. (2006) The effect of chronic administration of sarizotan, 5-HT_{1A} agonist/D₃/D₄ ligand, on haloperidol-induced repetitive jaw movements in rat model of tardive dyskinesia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 30(2): 273–279.
- Roth, B.L., Ciaranello, R.D. and Meltzer, H.Y. (1992) Binding of typical and atypical antipsychotic agents to transiently expressed 5-HT_{1C} receptors. *J. Pharmacol. Exp. Ther.*, 260(3): 1361–1365.
- Roth, B.L., Meltzer, H.Y. and Khan, N. (1998) Binding of typical and atypical antipsychotic drugs to multiple neurotransmitter receptors. *Adv. Pharmacol.*, 42: 482–485.
- Ruat, M., Traiffort, E., Arrang, J.M., Tardivel-Lacombe, J., Diaz, J., Leurs, R. and Schwartz, J.C. (1993a) A novel rat serotonin (5-HT₆) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.*, 193(1): 268–276.
- Ruat, M., Traiffort, E., Leurs, R., Tardivel-Lacombe, J., Diaz, J., Arrang, J.M. and Schwartz, J.C. (1993b) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT₇) activating cAMP formation. *Proc. Natl. Acad. Sci. U.S.A.*, 90(18): 8547–8551.
- Rueter, L.E., Tecott, L.H. and Blier, P. (2000) In vivo electrophysiological examination of 5-HT₂ responses in 5-HT_{2C} receptor mutant mice. *Naunyn Schmiedeberg Arch. Pharmacol.*, 361(5): 484–491.
- Samad, N., Khan, A., Perveen, T., Haider, S., Abdul Haleem, M. and Haleem, D.J. (2007) Increase in the effectiveness of somatodendritic 5-HT-1A receptors in a rat model of tardive dyskinesia. *Acta Neurobiol. Exp.*, 67(4): 389–397.
- Sari, Y., Miquel, M.C., Brisorgueil, M.J., Ruiz, G., Doucet, E., Hamon, M. and Verge, D. (1999) Cellular and subcellular localization of 5-hydroxytryptamine_{1B} receptors in the rat

- central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience*, 88(3): 899–915.
- Sawada, M., Nagatsu, T., Nagatsu, I., Ito, K., Iizuka, R., Kondo, T. and Narabayashi, H. (1985) Tryptophan hydroxylase activity in the brains of controls and parkinsonian patients. *J. Neural Transm.*, 62(1–2): 107–115.
- Scatton, B., Javoy-Agid, F., Rouquier, L., Dubois, B. and Agid, Y. (1983) Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. *Brain Res.*, 275(2): 321–328.
- Schiller, L., Jahkel, M., Kretschmar, M., Brust, P. and Oehler, J. (2003) Autoradiographic analyses of 5-HT_{1A} and 5-HT_{2A} receptors after social isolation in mice. *Brain Res.*, 980(2): 169–178.
- Scholtissen, B., Verhey, F.R., Steinbusch, H.W. and Leentjens, A.F. (2006) Serotonergic mechanisms in Parkinson's disease: opposing results from preclinical and clinical data. *J. Neural Transm.*, 113(1): 59–73.
- Schotte, A., Janssen, P.F., Megens, A.A. and Leysen, J.E. (1993) Occupancy of central neurotransmitter receptors by risperidone, clozapine and haloperidol, measured ex vivo by quantitative autoradiography. *Brain Res.*, 631(2): 191–202.
- Sethi, K.D. (2003) Tremor. *Curr. Opin. Neurol.*, 16(4): 481–485.
- Shen, K.Z. and Johnson, S.W. (2008) 5-HT inhibits synaptic transmission in rat subthalamic nucleus neurons in vitro. *Neuroscience*, 151(4): 1029–1033.
- Shen, K.Z., Kozell, L.B. and Johnson, S.W. (2007) Multiple conductances are modulated by 5-HT receptor subtypes in rat subthalamic nucleus neurons. *Neuroscience*, 148(4): 996–1003.
- Shilliam, C.S. and Dawson, L.A. (2005) The effect of clozapine on extracellular dopamine levels in the shell subregion of the rat nucleus accumbens is reversed following chronic administration: comparison with a selective 5-HT_{2C} receptor antagonist. *Neuropsychopharmacology*, 30(2): 372–380.
- Shink, E., Bevan, M.D., Bolam, J.P. and Smith, Y. (1996) The subthalamic nucleus and the external pallidum: two tightly interconnected structures that control the output of the basal ganglia in the monkey. *Neuroscience*, 73(2): 335–357.
- Sian, J., Gerlach, M., Youdim, M.B. and Riederer, P. (1999) Parkinson's disease: a major hypokinetic basal ganglia disorder. *J. Neural Transm.*, 106(5–6): 443–476.
- Simola, N., Morelli, M. and Carta, A.R. (2007) The 6-hydroxydopamine model of Parkinson's disease. *Neurotox. Res.*, 11(3–4): 151–167.
- Sivam, S.P., Breese, G.R., Krause, J.E., Napier, T.C., Mueller, R.A. and Hong, J.S. (1987) Neonatal and adult 6-hydroxydopamine-induced lesions differentially alter tachykinin and enkephalin gene expression. *J. Neurochem.*, 49(5): 1623–1633.
- Smith, I.D. and Grace, A.A. (1992) Role of the subthalamic nucleus in the regulation of nigral dopamine neuron activity. *Synapse*, 12(4): 287–303.
- Stachowiak, M.K., Bruno, J.P., Snyder, A.M., Stricker, E.M. and Zigmond, M.J. (1984) Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. *Brain Res.*, 291(1): 164–167.
- Stanford, I.M., Kantaria, M.A., Chahal, H.S., Loucif, K.C. and Wilson, C.L. (2005) 5-Hydroxytryptamine induced excitation and inhibition in the subthalamic nucleus: action at 5-HT_{2C}, 5-HT₄ and 5-HT_{1A} receptors. *Neuropharmacology*, 49(8): 1228–1234.
- Stanford, I.M. and Lacey, M.G. (1996) Differential actions of serotonin, mediated by 5-HT_{1B} and 5-HT_{2C} receptors, on GABA-mediated synaptic input to rat substantia nigra pars reticulata neurons in vitro. *J. Neurosci.*, 16(23): 7566–7573.
- Stefani, A., Fedele, E., Mazzone, P., Galati, S., Tropepi, P. and Stanzione, P. (2006) In vivo microdialysis in parkinsonian patients undergoing stereotactic neurosurgery: key insights on DBS mechanisms of actions. In: Valentini, V., Di Chiara, G. (Eds.), *Proceedings of the 11th International Conference of In Vivo Methods*, University of Cagliari, Sardinia, Italy, pp. 74–76.
- Stefani, A., Surmeier, D.J. and Kitai, S.T. (1990) Serotonin enhances excitability in neostriatal neurons by reducing voltage-dependent potassium currents. *Brain Res.*, 529(1–2): 354–357.
- Steffensen, S.C., Svingos, A.L., Pickel, V.M. and Henriksen, S.J. (1998) Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J. Neurosci.*, 18(19): 8003–8015.
- Steinbusch, H.W. (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*, 6(4): 557–618.
- Steward, L.J., Bufton, K.E., Hopkins, P.C., Davies, W.E. and Barnes, N.M. (1993) Reduced levels of 5-HT₃ receptor recognition sites in the putamen of patients with Huntington's disease. *Eur. J. Pharmacol.*, 242(2): 137–143.
- Sturman, M.M., Vaillancourt, D.E., Metman, L.V., Bakay, R.A. and Corcos, D.M. (2004) Effects of subthalamic nucleus stimulation and medication on resting and postural tremor in Parkinson's disease. *Brain*, 127(Pt 9): 2131–2143.
- Tarsy, D. and Baldessarini, R.J. (1984) Tardive dyskinesia. *Annu. Rev. Med.*, 35: 605–623.
- Tauscher, J., Kapur, S., Verhoeff, N.P., Hussey, D.F., Daskalakis, Z.J., Tauscher-Wisniewski, S., Wilson, A.A., Houle, S., Kasper, S. and Zipursky, R.B. (2002) Brain serotonin 5-HT_{1A} receptor binding in schizophrenia measured by positron emission tomography and [11C]WAY-100635. *Arch. Gen. Psychiatry*, 59(6): 514–520.
- Tepper, J.M. and Bolam, J.P. (2004) Functional diversity and specificity of neostriatal interneurons. *Curr. Opin. Neurobiol.*, 14(6): 685–692.
- Trevitt, J., Atherton, A., Aberman, J. and Salamone, J.D. (1998) Effects of subchronic administration of clozapine, thioridazine and haloperidol on tests related to extrapyramidal motor function in the rat. *Psychopharmacology (Berl.)*, 137(1): 61–66.
- Trevitt, J.T., Lyons, M., Aberman, J., Carriero, D., Finn, M. and Salamone, J.D. (1997) Effects of clozapine, thioridazine, risperidone and haloperidol on behavioral tests related to extrapyramidal motor function. *Psychopharmacology (Berl.)*, 132(1): 74–81.

- Twarog, B.M. and Page, I.H. (1953) Serotonin content of some mammalian tissues and urine and a method for its determination. *Am. J. Physiol.*, 175(1): 157–161.
- Ullmer, C., Engels, P., Abdel'Al, S. and Lubbert, H. (1996) Distribution of 5-HT₄ receptor mRNA in the rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 354(2): 210–212.
- Utter, A.A. and Basso, M.A. (2008) The basal ganglia: an overview of circuits and function. *Neurosci. Biobehav. Rev.*, 32(3): 333–342.
- Van Bockstaele, E.J., Biswas, A. and Pickel, V.M. (1993) Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Res.*, 624(1–2): 188–198.
- Van Bockstaele, E.J., Cestari, D.M. and Pickel, V.M. (1994) Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. *Brain Res.*, 647(2): 307–322.
- Van Bockstaele, E.J. and Pickel, V.M. (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.*, 682(1–2): 215–221.
- Vandermaelen, C.P., Bonduki, A.C. and Kitai, S.T. (1979) Excitation of caudate-putamen neurons following stimulation of the dorsal raphe nucleus in the rat. *Brain Res.*, 175(2): 356–361.
- Varnas, K., Hall, H., Bonaventure, P. and Sedvall, G. (2001) Autoradiographic mapping of 5-HT_{1B} and 5-HT_{1D} receptors in the post mortem human brain using [(3)H]GR 125743. *Brain Res.*, 915(1): 47–57.
- Varnas, K., Hurd, Y.L. and Hall, H. (2005) Regional expression of 5-HT_{1B} receptor mRNA in the human brain. *Synapse*, 56(1): 21–28.
- Varnas, K., Thomas, D.R., Tupala, E., Tiihonen, J. and Hall, H. (2004) Distribution of 5-HT₇ receptors in the human brain: a preliminary autoradiographic study using [(3)H]SB-269970. *Neurosci. Lett.*, 367(3): 313–316.
- Veazey, C., Aki, S.O., Cook, K.F., Lai, E.C. and Kunik, M.E. (2005) Prevalence and treatment of depression in Parkinson's disease. *J. Neuropsychiatry Clin. Neurosci.*, 17(3): 310–323.
- Verge, D., Daval, G., Marcinkiewicz, M., Patey, A., el Mestikawy, S., Gozlan, H. and Hamon, M. (1986) Quantitative autoradiography of multiple 5-HT₁ receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J. Neurosci.*, 6(12): 3474–3482.
- Vilario, M.T., Cortes, R., Gerald, C., Branchek, T.A., Palacios, J.M. and Mengod, G. (1996) Localization of 5-HT₄ receptor mRNA in rat brain by in situ hybridization histochemistry. *Brain Res. Mol. Brain Res.*, 43(1–2): 356–360.
- Vilario, M.T., Cortes, R. and Mengod, G. (2005) Serotonin 5-HT₄ receptors and their mRNAs in rat and guinea pig brain: distribution and effects of neurotoxic lesions. *J. Comp. Neurol.*, 484(4): 418–439.
- Waddington, J.L., Molloy, A.G., O'Boyle, K.M. and Mashurano, M. (1986) Motor consequences of D-1 dopamine receptor stimulation and blockade. *Clin. Neuropharmacol.*, 9(4): 20–22.
- Wadenberg, M.L. (1996) Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. *Neurosci. Biobehav. Rev.*, 20(2): 325–339.
- Waeber, C., Dietl, M.M., Hoyer, D. and Palacios, J.M. (1989) 5-HT₁ receptors in the vertebrate brain. Regional distribution examined by autoradiography. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 340(5): 486–494.
- Waeber, C., Schoeffter, P., Hoyer, D. and Palacios, J.M. (1990a) The serotonin 5-HT_{1D} receptor: a progress review. *Neurochem. Res.*, 15(6): 567–582.
- Waeber, C., Sebben, M., Nieoullon, A., Bockaert, J. and Dumuis, A. (1994) Regional distribution and ontogeny of 5-HT₄ binding sites in rodent brain. *Neuropharmacology*, 33(3–4): 527–541.
- Waeber, C., Zhang, L.A. and Palacios, J.M. (1990b) 5-HT_{1D} receptors in the guinea pig brain: pre- and postsynaptic localizations in the striatonigral pathway. *Brain Res.*, 528(2): 197–206.
- Ward, R.P. and Dorsa, D.M. (1996) Colocalization of serotonin receptor subtypes 5-HT_{2A}, 5-HT_{2C}, and 5-HT₆ with neuropeptides in rat striatum. *J. Comp. Neurol.*, 370(3): 405–414.
- Ward, R.P., Hamblin, M.W., Lachowicz, J.E., Hoffman, B.J., Sibley, D.R. and Dorsa, D.M. (1995) Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience*, 64(4): 1105–1111.
- Weiner, D.M., Vanover, K.E., Brann, M.R., Meltzer, H.Y. and Davis, R.E. (2003) Psychosis of Parkinson's disease: serotonin 2A receptor inverse agonists as potential therapeutics. *Curr. Opin. Investig. Drugs*, 4(7): 815–819.
- Wesolowska, A. (2002) In the search for selective ligands of 5-HT₅, 5-HT₆ and 5-HT₇ serotonin receptors. *Pol. J. Pharmacol.*, 54(4): 327–341.
- Wilms, K., Vierig, G. and Davidowa, H. (2001) Interactive effects of cholecystokinin-8S and various serotonin receptor agonists on the firing activity of neostriatal neurones in rats. *Neuropeptides*, 35(5–6): 257–270.
- Wolf, W.A., Bieganski, G.J., Guillen, V. and Mignon, L. (2005) Enhanced 5-HT_{2C} receptor signaling is associated with haloperidol-induced "early onset" vacuous chewing in rats: implications for antipsychotic drug therapy. *Psychopharmacology (Berl.)*, 182(1): 84–94.
- Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M. and Jennes, L. (1995) Comparative localization of serotonin 1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J. Comp. Neurol.*, 351(3): 357–373.
- Xiang, Z., Wang, L. and Kitai, S.T. (2005) Modulation of spontaneous firing in rat subthalamic neurons by 5-HT receptor subtypes. *J. Neurophysiol.*, 93(3): 1145–1157.
- Yakel, J.L., Trussell, L.O. and Jackson, M.B. (1988) Three serotonin responses in cultured mouse hippocampal and striatal neurons. *J. Neurosci.*, 8(4): 1273–1285.
- Yoshioka, M., Matsumoto, M., Togashi, H. and Mori, K. (1998) Central distribution and function of 5-HT₆ receptor subtype in the rat brain. *Ann. N. Y. Acad. Sci.*, 861(1): p. 244.

- Zhang, Q.J., Gao, R., Liu, J., Liu, Y.P. and Wang, S. (2007) Changes in the firing activity of serotonergic neurons in the dorsal raphe nucleus in a rat model of Parkinson's disease. *Sheng Li Xue Bao*, 59(2): 183–189.
- Zhang, X., Andren, P.E., Greengard, P. and Svenningsson, P. (2008) Evidence for a role of the 5-HT_{1B} receptor and its adaptor protein, p11, in L-DOPA treatment of an animal model of parkinsonism. *Proc. Natl. Acad. Sci. U.S.A.*, 105(6): 2163–2168.
- Zhang, X., Andren, P.E. and Svenningsson, P. (2007) Changes on 5-HT₂ receptor mRNAs in striatum and subthalamic nucleus in Parkinson's disease model. *Physiol. Behav.*, 92(1–2): 29–33.
- Zhou, F.C., Bledsoe, S. and Murphy, J. (1991) Serotonergic sprouting is induced by dopamine-lesion in substantia nigra of adult rat brain. *Brain Res.*, 556(1): 108–116.
- Zhou, F.M., Wilson, C.J. and Dani, J.A. (2002) Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J. Neurobiol.*, 53(4): 590–605.

CHAPTER 22

Serotonin–dopamine interaction in the induction and maintenance of L-DOPA-induced dyskinesias

Manolo Carta^{1,2,*}, Thomas Carlsson^{1,2}, Ana Muñoz¹, Deniz Kirik² and Anders Björklund¹

¹*Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden*

²*Brain Repair and Imaging in Neural Systems Unit, Section for Neuroscience, Department of Experimental Medical Science, Lund University, Lund, Sweden*

Abstract: Appearance of dyskinesia is a common problem of long-term Levodopa (L-DOPA) treatment in Parkinson's disease (PD) patients and represents a major limitation for the pharmacological management of the motor symptoms in the advanced stages of disease. An increasing body of evidence points to dopamine released as a false neurotransmitter from the striatal serotonin terminals as the main pre-synaptic determinant of L-DOPA-induced dyskinesia. Here we review the animal experimental and human clinical data in support of this view, which point to the serotonin system as a promising target for anti-dyskinetic therapy in PD patients under L-DOPA medication.

Keywords: L-DOPA; dopamine; serotonin; dyskinesia; 5-HT agonists; serotonin autoreceptors

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with an incidence of about 2% in the population over 60 years (Mayeux, 2003). The disease is characterized by a loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc), which in turn determines a decreased release of DA in the corpus striatum. The loss of DA input into the striatum is largely responsible for the motor symptoms, such as bradykinesia, postural instability, resting tremor and rigidity (Fahn, 2003).

Levodopa (L-DOPA) has been the first drug introduced in the clinical practice for the treatment of PD. Since DA cannot be administered directly, because it is unable to cross the blood brain barrier and reach the brain, its immediate precursor L-DOPA is given instead in combination with a peripheral decarboxylase inhibitor to avoid metabolism at blood-stream level. In this way, the striatal level of DA can be restored to physiological levels, accompanied by significant symptomatic effects.

L-DOPA is highly effective in relieving the motor symptoms during the first years of administration (the so-called 'honeymoon period'). However, over time, patients start experiencing side-effects, such as narrowing of the therapeutic window and appearance of dyskinesias, the most troublesome side-effect of L-DOPA medication.

*Corresponding author. Tel.: +46-46 22 20555;
Fax: +46-46 22 20559; E-mail: Manolo.Carta@med.lu.se

It has been reported that within 5 years about 50% of the patients develop these motor complications in response to L-DOPA administration (Obeso et al., 2000). This percentage rises to about 90% after the first decade (Ahlskog and Muenter, 2001). The appearance of dyskinesias represents, therefore, a serious limitation to the use of this therapeutic agent in advanced disease. Indeed, the only anti-dyskinetic drug currently available for patients is the glutamate receptor antagonist amantadine (Luginger et al., 2000). However, this compound has only a moderate efficacy, which declines over time and also induces side-effects (Crosby et al., 2003).

It is generally assumed that L-DOPA acts at the early stages of the disease by being taken up into the spared dopaminergic neurons and terminals, where it is converted to DA, stored into synaptic vesicles and released in a physiologically regulated manner. In this situation, a fine regulation of the neurotransmitter level in the synaptic cleft is assured by the presence of the D₂ autoreceptor and DA transporter. D₂ autoreceptors are important for the regulation of the firing pattern of DA neurons and therefore for the terminal release of DA; the DA transporter mediates the clearance of DA from the synaptic cleft back into DA terminals, assuring the maintenance of physiological levels of DA in the synapses and therefore a physiological stimulation of DA receptors located on the striatal dopaminergic neurons (Venton et al., 2003; Cragg and Rice, 2004). The feedback controlled mechanism of DA release from the spared DA terminals represents, therefore, an important element in providing the therapeutic efficacy of L-DOPA medication at early stages of the disease.

The serotonin system in L-DOPA-induced dyskinesias: evidence in rats

As the dopaminergic degeneration progresses, fewer and fewer DA terminals can contribute to the conversion of peripheral administered L-DOPA. In this scenario, other neuronal and non-neuronal cell types are suggested to play a role in DA production. Among these cells, serotonergic

neurons represent an interesting element because they express the amino acid aromatic decarboxylase (AADC) and the vesicular monoamine transporter 2 (VMAT2), which are responsible for the conversion of L-DOPA to DA and storage of DA into synaptic vesicles, respectively. A number of experimental paradigms have indeed demonstrated the ability of the serotonergic neurons to store and release DA both in vivo and in vitro (Ng et al., 1970, 1971; Hollister et al., 1979; Lavoie and Parent, 1990; Arai et al., 1994, 1995, 1996; Nicholson and Brotchie, 2002; Maeda et al., 2005). In line with these reports, Tanaka et al. (1999) have shown that lesion of the serotonin system by the specific toxin 5,7-dihydroxytryptamine (5,7-DHT), reduced L-DOPA-derived extracellular DA by about 80% in hemiparkinsonian rats. The same group showed a similar reduction in extracellular DA level after co-administration of the 5-HT_{1A} agonist 8-OHDPAT with L-DOPA (Kannari et al., 2001). These changes were paralleled by the reduced up-regulation of dynorphin and glutamic acid decarboxylase in the DA-denervated striatum, both of which are established markers of L-DOPA-induced motor complications. Similar results were obtained by Lopez et al. (2001), who showed reduced rotational behaviour and c-fos expression after removal of the serotonin system in 6-OHDA-lesioned rats administered with 30 mg/kg L-DOPA. Interestingly, Kannari et al. (2000) have demonstrated that L-DOPA-derived DA release is highly reduced, in the dopaminergic denervated striatum, when vesicular storage is blocked by reserpine pretreatment. Reserpine is able to deplete vesicular storage of amines by binding to VMAT (Peter et al., 1994); in this condition, L-DOPA-derived DA release was reduced by more than 80% in the latter study. These results suggest that, in the DA denervated striatum, L-DOPA-derived extracellular DA derives to a large degree from vesicular storage and it is released by an exocytosis mechanism, by the striatal serotonin terminals.

In addition to the serotonin system, glial cells also possess AADC activity and may contribute to DA production after L-DOPA administration (Melamed et al., 1981; Mura et al., 1995; Lopez-Real et al., 2003). Indeed, Lopez and colleagues

reported a re-emergence of the rotational behaviour in rats treated with very high-doses of L-DOPA (100 mg/kg) (Lopez et al., 2001). A similar observation was recently made by Carta et al. (2007) who reported partial re-appearance of dyskinesias in serotonin lesioned rats after administration of L-DOPA at a high-dose (Carta et al., 2007). However, these doses far exceed the ones used in humans, and the physiological relevance of this non-neuronal contribution remains unclear.

The susceptibility to dyskinesia increases over time in PD patients as neurodegeneration progresses. This raises the possibility that exogenous DA is released from non-dopaminergic cells in a non-physiological, dysregulated manner. Carta et al. (2007) have recently shown that DA released from the serotonin system is responsible for the appearance of L-DOPA-induced dyskinesias in rats where the intrinsic striatal dopaminergic innervation is removed by 6-OHDA. In this set of experiments, toxic lesion of the serotonin system by 5,7-DHT, or pharmacological silencing of these neurons by selective 5-HT_{1A} and 5-HT_{1B} agonists, produced a near-complete abolishment of dyskinesias induced by repetitive daily injection of L-DOPA (at 6 mg/kg dose) in the rat 6-OHDA model.

The serotonergic neurons express three subtypes of autoreceptors, among which the 5-HT_{1A} and 5-HT_{1B} are most abundant. 5-HT_{1A} receptors are present at the cell body level, as well as on the dendrites, in the dorsal and median raphe nuclei, where they regulate the firing of the serotonin neurons (Chalmers and Watson, 1991; Riad et al., 2000). The 5-HT_{1B} receptors, by contrast, are more abundant at the terminal-level in the areas innervated by the serotonin system, including the striatum, where they serve to control the terminal release of the neurotransmitter (Sari et al., 1999; Knobelmann et al., 2000; Adell et al., 2001). Together these two main classes of autoreceptors are able to fine-tune the synaptic release of serotonin in the forebrain target regions, keeping the synaptic level of this neurotransmitter within a physiological level (Sprouse and Aghajanian, 1987). In line with these data, drugs, such as the 5-HT_{1A} agonist 8-OHDPAT and the 5-HT_{1B} agonist CP-94253, which act as serotonin

autoreceptor agonists, have been shown to decrease the synaptic release of serotonin (Sprouse and Aghajanian, 1987; Knobelmann et al., 2000; Riad et al., 2000; Adell et al., 2001).

It is conceivable that serotonin autoreceptor agonists, by dampening the release of serotonin, would also decrease the release of L-DOPA-derived DA from the serotonin terminals, since the two transmitters are co-localized in the same synaptic vesicles after exogenous administration of L-DOPA (Arai et al., 1994, 1995, 1996). Interestingly, Carta et al. (2007) have found a potent synergistic effect between the 5-HT_{1A} agonist (\pm)-8-OHDPAT and the 5-HT_{1B} agonist CP-94253 in blocking L-DOPA-induced dyskinesia in Parkinsonian rats. Thus, sub-threshold doses of the two compounds, which individually produced either no, or only a mild effect completely suppressed dyskinesia when administered together. Possibly, the differential location of the two autoreceptors on the serotonergic neurons plays an important role in the manifestation of this synergistic effect. Regardless of the underlying mechanism, this synergistic action might have interesting clinical applications and deserves further investigation. Furthermore, the finding of a complete suppression of L-DOPA-induced dyskinesias by a combination of low-doses of 5-HT_{1A} and 5-HT_{1B} agonists has identified the serotonin system as the unique pre-synaptic determinant of L-DOPA-induced dyskinesia in Parkinsonian rats. In contrast, previous studies targeting either one or the other autoreceptor have shown only a partial reduction of the abnormal movements (Bibbiani et al., 2001; Jackson et al., 2004; Iravani et al., 2006).

A recent report by Carlsson et al. (2007) has provided further support for the involvement of the serotonin system in L-DOPA-induced dyskinesias in rats (Carlsson et al., 2007). In this study, the authors have used transplants of foetal serotonin neurons to provide a serotonergic hyper-innervation of the DA-denervated striatum. In these animals, dyskinesias induced by a single dose of L-DOPA (6 mg/kg) in already primed animals were increased by up to 70% compared to the pre-transplantation score. Foetal DA neuron grafts, by contrast, were able to significantly reduce the abnormal movements (by about 60%),

thus adding further evidence for the requirement of a feedback control mechanism of the synaptic release of DA to avoid uncontrolled swings in DA levels.

In the animal model used in the latter and other studies, abnormal involuntary movements are produced in hemiparkinsonian rats upon chronic treatment with low-L-DOPA doses (6–12 mg/kg per day), resembling peak-dose dyskinesias seen in PD patients (Cenci et al., 1998; Lundblad et al., 2002, for further details). This model has been used to investigate molecular changes associated with the appearance of dyskinesias, such as up-regulation of striatal peptides, pro-dynorphin and pre-pro-enkephalin in particular, as well as intracellular signalling pathways involved in these changes in striatal dopaminergic neurons (Cenci et al., 1998; Andersson et al., 1999; Westin et al., 2001; Santini et al., 2007). Many of these changes resemble the ones found in PD patients. As a further validation of the model, drugs proven to have anti-dyskinetic properties in patients also reduce abnormal movements in rats and mice (Lundblad et al., 2002, 2005). This model represents, therefore a unique opportunity not only to identify better compounds that may help in the management of L-DOPA-induced dyskinesias, but also to investigate changes in striatal cellular function induced by dopaminergic denervation.

In line with these findings, using the same animal model, Eskow et al. (2007) have recently shown that the partial 5-HT_{1A} agonist buspirone reduces development and expression of L-DOPA-induced dyskinesias. Furthermore, Carta and co-workers have demonstrated the ability of exogenous L-DOPA to displace serotonin from the striatal tissue, confirming earlier observations in mice with high-L-DOPA doses (Everett and Borcharding, 1970; Carta et al., 2007). This phenomenon, which is probably due to the competition between L-DOPA-derived DA and serotonin for storage into the synaptic vesicles in serotonin terminals, is likely to induce an over-activation of the serotonin neurons in order to compensate for the reduced binding of serotonin to its autoreceptors. This over activation, in turn, would further add to the excessive release of DA from the serotonin neurons, thus contributing to

the high-swings in synaptic DA levels associated with dyskinesias. Taken together, these results provide evidence that the serotonergic system plays a pivotal role in induction of dyskinesias by L-DOPA in the rat PD model.

A pre-synaptic model of L-DOPA-induced dyskinesia

In line with these observations, a pre-synaptic model of L-DOPA-induced dyskinesias is proposed in Fig. 1. In this model, the full therapeutic effect of L-DOPA would be maintained as long as there are enough spared striatal DA terminals to mediate conversion of L-DOPA and release of DA in a physiologically controlled manner. In this situation, adequate regulation of the level of the neurotransmitter in the synaptic cleft would be assured by the presence of D₂ receptors and DA transporters on the DA terminals. As the neuro-degeneration progresses, however, the serotonin system would emerge as the prime site of L-DOPA conversion. In fact, the presence of AADC and VMAT2 in serotonergic neurons makes it possible for L-DOPA-derived DA to be formed, stored and released along with serotonin, thus acting as a ‘false transmitter’. Although the serotonin neurons are able to mediate the vesicular release of DA in an activity-dependent manner, the same neurons cannot regulate the synaptic level of DA due to the absence of the pre-synaptic machinery expressed by the DA neurons. This misbalance between the releasing ability of serotonin terminals for exogenous DA and the lack of a feedback control mechanism, would be responsible for the excessive synaptic swings in DA levels, which have been suggested to cause the abnormal movements (Chase, 1998; Olanow and Obeso, 2000; de la Fuente-Fernandez et al., 2004). In addition, depletion of striatal serotonin content by L-DOPA administration would further add to the high-synaptic level of DA by inducing over-activity of the serotonin neurons. As predicted by this model, activation of the 5-HT_{1A} and 5-HT_{1B} autoreceptors by agonist drugs, which is known to reduce the activity of serotonin neurons and inhibit the synaptic release of the neurotransmitter, was able

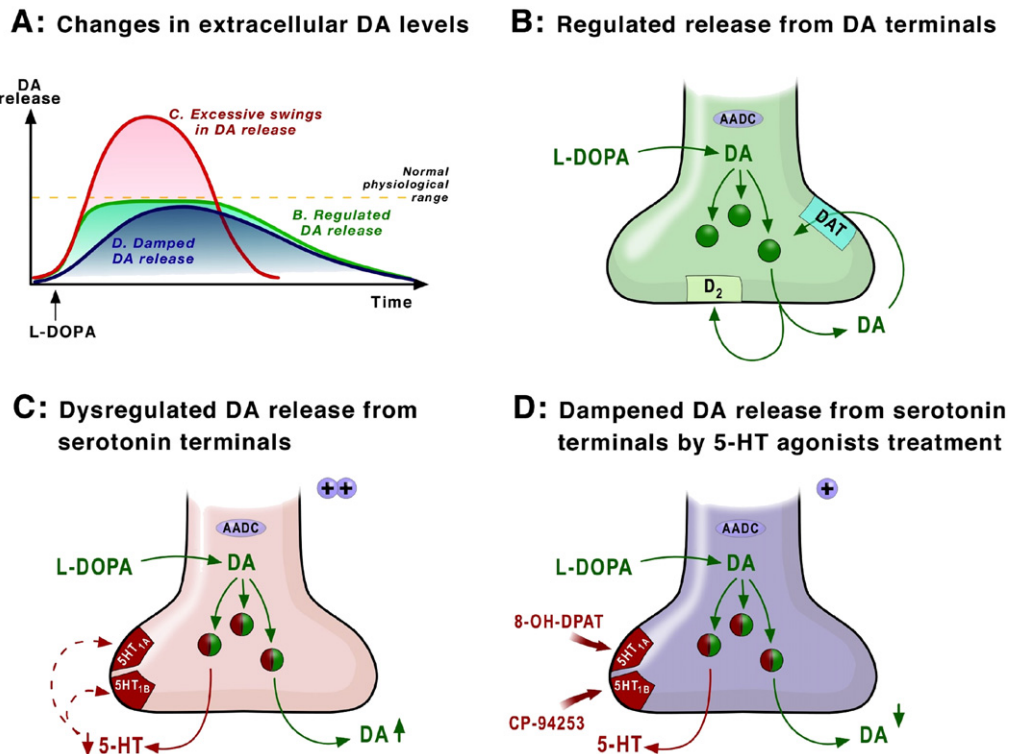


Fig. 1. The figure shows a pre-synaptic model of L-DOPA-induced dyskinesias. In early stages of PD (panel B), the therapeutic effect of L-DOPA is sustained by the spared striatal DA terminals. At this stage of the disease, L-DOPA is taken up by the DA terminals, stored into vesicles and released in an activity-dependent manner. Excessive swings in extracellular DA levels are prevented by auto-regulatory feedback mechanism mediated by the DA transporter and the D₂ autoreceptors present on the dopaminergic terminals (green line in panel A). As neurodegeneration progresses, fewer and fewer DA terminals will remain to mediate L-DOPA conversion and the serotonin terminals will come to play a major role (panel C). However, due to the lack of normal auto-regulatory feedback control and concomitant hyper-activation of serotonin terminals caused by depletion of endogenous serotonin by DA accumulating in the storage vesicles, DA released from serotonin terminals will be poorly regulated, resulting in uncontrolled, excessive swings in DA release (red line in panel A). The imbalance between the capacity of the serotonin terminals to release L-DOPA-derived DA, and the inability of the same neurons to provide a feedback control mechanism to regulate the level of the neurotransmitter in the synaptic cleft, would be the driving force in the induction of dyskinesia. According to this model, 5-HT_{1A} and 5-HT_{1B} agonists, particularly in combination, completely suppress L-DOPA-induced abnormal movements in 6-OHDA-lesioned rats by dampening synaptic DA levels to a more physiological range (panel D and blue line in panel A). Note that for simplicity 5-HT_{1A} receptors are positioned at the terminal level, but are indeed located at the level of the cell body of serotonin neurons. (See Color Plate 22.1 in color plate section.)

to completely abolish L-DOPA-induced abnormal involuntary movements (Carta et al., 2007).

In agreement with this, de la Fuente-Fernandez et al. (2004) have shown in a recent PET imaging study that peak-dose dyskinesias in advanced PD patients is associated with excessive swings in synaptic DA after oral L-DOPA administration (de la Fuente-Fernandez et al., 2004). These

synaptic swings, in turn, would determine a 'pulsatile' stimulation of DA receptors on the dopaminoceptive striatal neurons, which has been suggested to play an important role in the emergence of motor complications associated with L-DOPA medication. In fact, more continuous administration of L-DOPA or DA agonists, such as duodenal or subcutaneous infusion by pumps,

are less prone to induce these side-effects (Olanow and Obeso, 2000; Olanow et al., 2000; Nyholm and Aquilonius, 2004). Oral intermittent administration of L-DOPA and dysregulated DA release from the serotonin terminals would act cooperatively in the development of dyskinetic movements and in the induction of post-synaptic changes in the dopaminergic striatal neurons. In fact, alterations in the D₁ signalling pathway and changes in NMDA (*N*-methyl-D-aspartic acid) and AMPA receptor function and distribution are known to play an important role in the induction and maintenance of abnormal movements in rodent and non-human primate models of PD (Dunah et al., 2000; Dunah and Standaert, 2001; Lin et al., 2003; Picconi et al., 2003; Dunah et al., 2004; Robelet et al., 2004; Aubert et al., 2005; Hallett et al., 2005; Fiorentini et al., 2006; Gardoni et al., 2006; Guigoni et al., 2007; Picconi et al., 2007; Santini et al., 2007). These post-synaptic alterations are accompanied by long-lasting changes in gene expression, which are a characteristic feature of these motor complications (Cenci et al., 1998; Andersson et al., 1999).

The serotonin system in L-DOPA-induced dyskinesias: observations in MPTP-lesioned monkeys

MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) is a chemical related to the opioid analgesic drugs. MPTP itself does not have any hedonic effect, but it may be produced accidentally during illicit manufacture of MPPP (1-methyl-4-phenyl-4-propionoxypiperidine), a synthetic opioid with effects similar to those of heroin and morphine. MPTP is metabolized into MPP⁺ by MAO-B, which interferes with complex I of the electron transport chain, a component of mitochondrial metabolism, causing selective cell death of the dopaminergic neurons in the SNc (Langston et al., 1984b). Langston and co-workers were the first to report that injections of MPTP in squirrel monkeys result in Parkinsonian like symptoms, due to selective degeneration of the DA neurons (Langston and Ballard, 1984; Langston et al., 1984a, c).

To date, MPTP-treated monkeys represent the most accepted model of human PD and share several features with the disease in humans. Whether the serotonergic system plays a similar role in DA production and L-DOPA-induced dyskinesia in the monkey model as the one described above in rats, is still an open issue. However, in support of this view, Iravani et al. (2006) have recently reported a partial reduction of L-DOPA-induced dyskinesia in MPTP-treated marmosets using the 5-HT_{1A} agonist (+)-8-OHD-PAT. In addition, the same authors have previously shown a similar partial effect using a 5-HT_{1B/1D} agonist (Jackson et al., 2004). As mentioned previously, drugs targeting the 5-HT_{1A} and the 5-HT_{1B} autoreceptors are effective in reducing the release of serotonin and can also dampen the release of L-DOPA-derived DA from the serotonin neurons. In line with the results obtained in rodents, therefore, these observations point to the serotonergic system as a key element in DA production and induction of dyskinesia also in the primate model of PD. In these studies, however, relatively high doses of the agonists were used and side-effects, such as worsening of Parkinsonism, were reported (Iravani et al., 2006). Conversely, Bibbiani et al. (2001) have shown that the 5-HT_{1A} partial agonist Sarizotan can reduce dyskinesia without any significant worsening of the Parkinsonian symptoms (Bibbiani et al., 2001). This discrepancy might be ascribed to differences in the action profile of the compounds used or to differences in the magnitude of MPTP-induced DA depletion in the two studies. Indeed, different MPTP lesion protocols can result in a different degree of DA depletion. Thus, preservation of a partial DA innervation can have profound consequences on the therapeutic effect of L-DOPA when DA release from serotonin neurons is silenced. In animals with an incomplete lesion, the spared DA terminals can serve to buffer the availability of L-DOPA after intermittent administration and mediate a physiological, feedback regulated release of DA, thus preventing the induction of excessive synaptic swings in DA levels and the appearance of dyskinesias. In the presence of complete dopaminergic depletion, in contrast, the main source of extracellular DA from

exogenous L-DOPA would derive from the serotonin neurons. In such a scenario, a complete silencing of DA release from the serotonin terminals can be expected to diminish not only dyskinesias, but also any therapeutic effect derived from this source of DA, thus providing a possible explanation for the worsened therapeutic response to L-DOPA reported by Iravani et al. (2006) in the (+)-8-OHDPAT-treated animals. Special attention therefore should be paid to the experimental conditions in which these therapeutic agents are tested.

Reduced production of DA, however, is unlikely to be the only explanation for the diminished therapeutic efficacy of L-DOPA reported by Iravani and co-workers after co-administration with (+)-8-OHDPAT. In this study in fact, the authors observed a similar reduction in the anti-Parkinsonian effect of the D2/3 direct agonist pramipexole after co-administration with (+)-8-OHDPAT. The decreased anti-Parkinsonian effect of L-DOPA might therefore be due, at least in part, also to the high dose of 5-HT_{1A} agonists necessary to obtain a significant anti-dyskinetic effect, when given individually. High doses of 5-HT_{1A} agonists are known to activate not only the pre-synaptic receptors, but also the receptors located post-synaptically on other neuronal types, such as the cortical glutamatergic neurons (Ceci et al., 1994; Casanovas et al., 1999; Hajos et al., 1999; Antonelli et al., 2005; Mignon and Wolf, 2005). In rodents, activation of post-synaptic 5-HT_{1A} receptors have been linked to the so-called serotonin syndrome, which is characterized by flat body posture, reciprocal forepaw treading and head weaving (Goodwin et al., 1986; Smith and Peroutka, 1986; Yamada et al., 1988; Hoyer et al., 2002; Carey et al., 2004). Iravani and co-workers observed a similar behaviour after administration of 8-OHDPAT in MPTP-lesioned marmosets, which may have contributed to the appearance of hypokinesia and dystonia in their animals. Appearance of these side effects after pharmacological targeting of serotonin receptors might represent a serious concern for a possible clinical application of serotonin agonists as anti-dyskinetic agents. For this reason, further investigations are needed in order to clarify whether these side effects

are due to intrinsic properties of the compounds employed, or to the high doses necessary to achieve a significant anti-dyskinetic effect when the drugs are used individually, and/or to the experimental conditions in which these studies have been performed (i.e., extent of DA depletion). The potent synergistic effect between 5-HT_{1A} and 5-HT_{1B} agonists might provide a solution to this problem. According to the rodent data, co-activation of these two autoreceptors at doses that are below the ones needed to provide significant activation of the post-synaptic receptors should be able to dampen the serotonin neuron release in the absence of any significant post-synaptic effect. Thus, if the motor complications seen in the Iravani study were due to the post-synaptic receptor activation, rather than due to the silencing effect on the serotonin neurons, this approach should be able to block the induction of dyskinesia without any interference with the anti-Parkinsonian properties of L-DOPA. Further studies are necessary to investigate this hypothesis.

The serotonin system in L-DOPA-induced dyskinesias: the human data

Based on positive results obtained in pre-clinical studies in both rats and monkeys, the 5-HT_{1A} partial agonist Sarizotan has been tested as an anti-dyskinetic agent in human clinical trials. Olanow et al. (2004) performed a first investigation in an open-label, multi-centre, dose escalation study to provide preliminary information on the safety, tolerability and efficacy of Sarizotan in patients with advanced PD and troublesome L-DOPA-induced dyskinesia. The study comprised a 3-week titration phase, 9-week maintenance phase and a 2-week withdrawal. Sarizotan treatment was initiated at a dose of 2mg twice daily during week 1, increased to 5mg twice daily in week 2 and titrated to a maximal dose of 10mg twice daily during week 3, depending on the response and tolerability. During the 9-week maintenance phase the dose was reduced in some of the patients due to worsening of Parkinsonism or other side effects. Sixty-four patients were employed in the study.

Mean disease duration and dyskinesia were 12.9 ± 5.0 and 5.7 ± 4.9 years, respectively. Fifty-one subjects completed the study, while 13 were lost due to adverse effect, treatment failure or withdrawal of consent. The treatment was associated with a significant increase in percent *on* time without dyskinesia during the waking day, which increased from a mean of 3.7 to 6.0 h. In parallel, *on* time with troublesome dyskinesia decreased from a mean of 4.5 to 2.5 h at the final treatment visit. The percentage of patients with moderate or severe dyskinesia was decreased from 81.2 to 38.5%. On the clinical global impression scale, 70.9% and 69% were considered to be improved in comparison with baseline by investigator and patients, respectively. The most common adverse effect related to Sarizotan was worsening of Parkinsonism, which was partly resolved by reducing the dose. The mean total daily dose at the final treatment visit was 8.9 ± 5.2 mg. Overall, in this study, Sarizotan resulted in significant reduction of dyskinesia, particularly of troublesome dyskinesia.

In the first double-blind, placebo controlled, proof-of-concept study, Bara-Jimenez et al. (2005) have investigated the effect of Sarizotan, given orally at 2 and 5 mg twice daily, in 18 relatively advanced PD patients (symptom duration 10 ± 4 years; L-DOPA treatment duration 6.1 ± 3.0 years). The authors reported a dose-dependent decrease in the dyskinesia score, which was statistically significant at 5 mg. This treatment prolonged the duration of the anti-Parkinsonian action of L-DOPA without diminishing the therapeutic efficacy. Although the extent of the anti-dyskinetic effect was less than in Parkinsonian monkeys treated with a similar dose, safety observations and the apparent dose-response effect suggested that higher doses might have proven more beneficial in this study. These results are consistent with a participation of vesicular storage and release of DA in human striatal serotonergic terminals, supporting the pre-clinical data discussed above.

Recently, the results of a larger multi-centre, randomized, placebo-controlled, double-blind, parallel study, designed to investigate the efficacy of Sarizotan on L-DOPA-induced dyskinesias have been reported by Goetz et al. (2007). Three

hundred and eighty one patients were included in this study and 338 completed the full programme of 12 weeks. Mean disease duration was 13.2 years and dyskinesias had been present for a mean of 5.1 years. Three doses of 2, 4 and 10 mg/day given in two doses were evaluated. Although mean improvements from 1.7 to 1.9 h in *on* time without dyskinesias, were observed with Sarizotan, these differences were not significantly different from the placebo-treated patients. Significant improvements with Sarizotan (2 mg/day) were found when considering item 32 and 33 in the unified Parkinson's disease rating scale, which evaluate dyskinesia duration and disability. Treatments with 4 and 10 mg/day were less favourable since they were associated with increased *off* time and did not provide any additional anti-dyskinetic effect.

Based on these results, a large phase III clinical trial was designed in order to investigate the efficacy of Sarizotan at the lowest dose, 2 mg/day, on dyskinesia in advanced PD patients. However, despite the promising results of the earlier reports, this trial was terminated due to lack of efficacy (Merck web site at <http://media.merck.de>). Although a detailed report of the phase III trial has not been published yet, there are several reasons that may account for the failure to obtain any significant anti-dyskinetic effect in this trial. First, the dose of drug chosen for this trial might have been too low to provide the necessary blockade of DA release from the serotonin terminals. Second, Sarizotan has also some antagonistic properties on the DA receptors, which might explain in part the side effects observed in the trial, particularly the worsening of Parkinsonism. However, in light of the pre-clinical results discussed above, and assuming that the serotonin terminals play a similar role in mediating L-DOPA-derived DA release in humans, it is also possible that targeting the 5-HT_{1A} receptors alone is not sufficient to provide a significant control of the excessive swings in DA release and therefore of dyskinesias. In light of the rat data, simultaneous activation of the 5-HT_{1A} and 5-HT_{1B} receptors might result in a more potent effect and in a better control of the motor side effects of L-DOPA medication.

It should also be noted that in advanced disease, where most of the dopaminergic terminals have degenerated, the serotonin system is likely to provide the main source of DA production and release in PD patients also. In this situation, any treatment that reduces the release of DA from serotonin terminals would affect not only dyskinesias, but also the anti-Parkinsonian therapeutic effect derived from this source of DA. Thus, it is possible that patients who retain some residual DA innervation, which can mediate L-DOPA-derived DA production and sustain the therapeutic effect of the drug, might benefit more from a treatment that silences the serotonin neurons. In such cases even a complete blockade of DA release from the serotonin terminals should not have a major impact on the therapeutic effect of L-DOPA. If this is correct, careful selection of patients, for instance by PET imaging, would be necessary in order to ensure maximal benefit from the serotonin agonist treatment.

Post-synaptic alterations in L-DOPA-induced dyskinesia

Intermittent oral administration of L-DOPA and dysregulated release of DA from striatal serotonin terminals are likely to cooperate in determining un-physiological stimulation of DA receptors located on the striatal dopaminergic neurons. This abnormal activation would be the triggering element of post-synaptic maladaptive changes that have been linked to dyskinesia, such as altered D1 and glutamate receptor transmission (Picconi et al., 2003, 2007; Robelet et al., 2004; Aubert et al., 2005; Guigoni et al., 2007; Santini et al., 2007). Indeed, normal D1 receptor function is required to regulate the trafficking of the NMDA receptor sub-units between different cellular compartments (Dunah and Standaert, 2001; Dunah et al., 2004). DA denervation has been shown to induce alterations in sub-unit composition of the NMDA receptors at the level of cortico-striatal synapse (Dunah et al., 2000; Bibbiani et al., 2005; Hallett et al., 2005; Fiorentini et al., 2006; Gardoni et al., 2006). These alterations, in turn, result in an abnormal re-distribution of these sub-units

between synaptic and extra-synaptic sites, which affects signalling cascades regulating gene expression. Gardoni et al. (2006) have shown that altered re-distribution of the NR2B sub-units of the NMDA receptor between the synaptic and extra-synaptic striatal membrane is associated with the appearance of L-DOPA-induced dyskinesias in 6-OHDA-lesioned rats. Increased extra-synaptic expression of NR2B sub-units is likely to result in reduced striatal glutamate-dependent activation of CREB, a transcription factor regulating gene expression. Furthermore, it has been shown that normal D1 and NMDA receptor activation are mandatory for physiological induction of striatal long-term potentiation (LTP) and long-term depression (LTD), electrophysiological events that are believed to underlie learning and memory processes (Calabresi et al., 2000, 2007; Pisani et al., 2005). Accordingly, DA denervation impairs striatal LTP, and L-DOPA treatment restores this form of synaptic plasticity in non-dyskinetic rats. Dyskinetic rats, by contrast, lack the ability to reverse previously induced LTP upon low-frequency stimulation of cortico-striatal afferents (Picconi et al., 2003, 2007). This lack of depotentiation has been associated with abnormal storage of motor information and inability of striatal neurons to discriminate between relevant and irrelevant cortical commands during neural coding of motor sequences. Eventually, these alterations have been proposed to result in changes in gene expressions at the level of striatal neurons, which are ultimately responsible for manifestation of the abnormal motor behaviour (Cenci et al., 1998; Andersson et al., 1999; Santini et al., 2007). In fact, up-regulation of genes coding for the peptides dynorphin and enkephalin, and the transcription factors fosB/ Δ fosB, are validated markers for L-DOPA-induced dyskinesia (Fig. 2 for a schematic representation of these alterations). In line with the proposed role of the NR2B sub-unit of the NMDA receptor in the induction of dyskinesia, this sub-unit has been identified as a promising target for anti-dyskinetic therapies acting post-synaptically to block expression of dyskinesias in both rodents and non-human primates models of PD (Hadj Tahar et al., 2004; Morissette et al., 2006a, b).

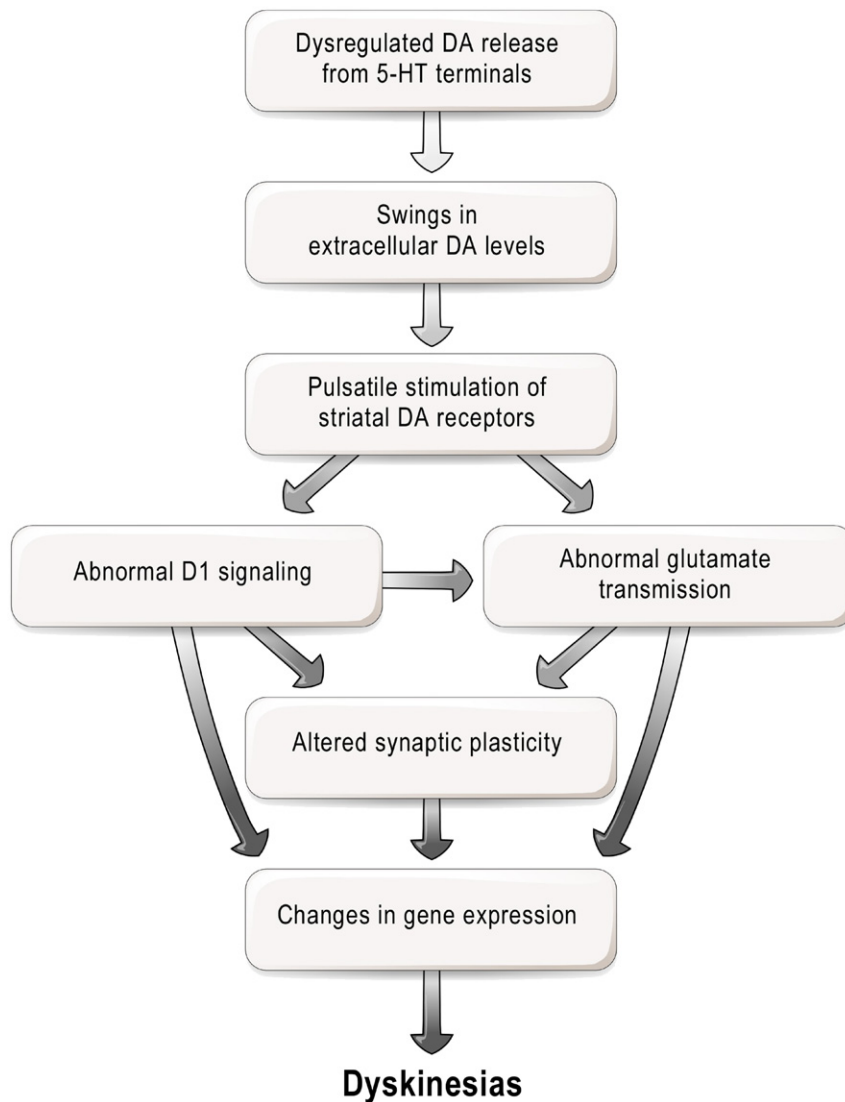


Fig. 2. The figure shows a schematic representation of changes associated with the dysregulated DA release from serotonin terminals. Dysregulated DA release from serotonin terminals, due to the lack of a feedback control mechanism, as illustrated in Fig. 1, is responsible for induction of excessive synaptic swings in striatal DA levels. Such synaptic swings would in turn contribute to the pulsatile stimulation of the post-synaptic striatal DA receptors. DA denervation and un-physiological DA receptor stimulation will trigger the alterations in D1 and glutamate signalling pathways associated with dyskinesia (see text). Cross-talk between these two types of receptor in the regulation of synaptic plasticity and gene expression induces a cascade of cellular events that eventually results in appearance of abnormal involuntary movements.

Conclusions

Appearance of dyskinesias remains a major problem for the pharmacological management of motor symptoms in PD patients. The only

anti-dyskinetic drug currently in use in clinical practice is the glutamate receptor antagonist amantadine, which provides, however, only modest and time-limited benefit. The progression of DA neurodegeneration paralleled by the emerging

role of the serotonin neurons in the conversion of L-DOPA appears to be the triggering element for the emergence of motor complications. Indeed, an increasing body of evidence points to DA released as a false neurotransmitter from the striatal serotonin terminals as the main pre-synaptic determinant of L-DOPA-induced dyskinesia in animal models of PD. Accordingly, 5-HT_{1A} and 5-HT_{1B} receptor agonists, particularly in combination, have been shown to be highly effective in counteracting L-DOPA-induced dyskinesias in the rat model. Provided that further evidence for the efficacy of this combined 5-HT_{1A/1B} agonist approach can be obtained in MPTP-treated monkeys, it would be intriguing to investigate the efficacy of the treatment in PD patients. Despite the failure of the recent clinical trial investigating the efficacy of Sarizotan on L-DOPA-induced dyskinesia, serotonin autoreceptor agonists remain promising therapeutic agents to control the most troublesome side effect of L-DOPA medication, L-DOPA-induced dyskinesias.

Acknowledgements

The author's work in this field is supported by grants from the Michael J. Fox Foundation (Community Fast Track 2005), from the Swedish Research Council (04X-3874 to AB) and from Parkinsonfonden. Manolo Carta is supported by the Parkinson's Disease Foundation (IRGP 2006 and 2007) and Michael J. Fox Foundation.

References

- Adell, A., Celada, P. and Artigas, F. (2001) The role of 5-HT_{1B} receptors in the regulation of serotonin cell firing and release in the rat brain. *J. Neurochem.*, 79: 172–182.
- Ahlskog, J.E. and Muenter, M.D. (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov. Disord.*, 16: 448–458.
- Andersson, M., Hilbertson, A. and Cenci, M.A. (1999) Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. *Neurobiol. Dis.*, 6: 461–474.
- Antonelli, T., Fuxe, K., Tomasini, M.C., Bartoszyk, G.D., Seyfried, C.A., Tanganelli, S. and Ferraro, L. (2005) Effects of sarizotan on the corticostriatal glutamate pathways. *Synapse*, 58: 193–199.
- Arai, R., Karasawa, N., Geffard, M. and Nagatsu, I. (1995) L-DOPA is converted to dopamine in serotonergic fibers of the striatum of the rat: a double-labeling immunofluorescence study. *Neurosci. Lett.*, 195: 195–198.
- Arai, R., Karasawa, N., Geffard, M., Nagatsu, T. and Nagatsu, I. (1994) Immunohistochemical evidence that central serotonin neurons produce dopamine from exogenous L-DOPA in the rat, with reference to the involvement of aromatic L-amino acid decarboxylase. *Brain Res.*, 667: 295–299.
- Arai, R., Karasawa, N. and Nagatsu, I. (1996) Aromatic L-amino acid decarboxylase is present in serotonergic fibers of the striatum of the rat. A double-labeling immunofluorescence study. *Brain Res.*, 706: 177–179.
- Aubert, I., Guigoni, C., Hakansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B.H., Gross, C.E., Fisone, G., Bloch, B. and Bezard, E. (2005) Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Ann. Neurol.*, 57: 17–26.
- Bara-Jimenez, W., Bibbiani, F., Morris, M.J., Dimitrova, T., Sherzai, A., Mouradian, M.M. and Chase, T.N. (2005) Effects of serotonin 5-HT_{1A} agonist in advanced Parkinson's disease. *Mov. Disord.*, 20: 932–936.
- Bibbiani, F., Oh, J.D. and Chase, T.N. (2001) Serotonin 5-HT_{1A} agonist improves motor complications in rodent and primate Parkinsonian models. *Neurology*, 57: 1829–1834.
- Bibbiani, F., Oh, J.D., Kielaite, A., Collins, M.A., Smith, C. and Chase, T.N. (2005) Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. *Exp. Neurol.*, 196: 422–429.
- Calabresi, P., Gubellini, P., Centonze, D., Picconi, B., Bernardi, G., Chergui, K., Svenningsson, P., Fienberg, A.A. and Greengard, P. (2000) Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. *J. Neurosci.*, 20: 8443–8451.
- Calabresi, P., Picconi, B., Tozzi, A. and Di Filippo, M. (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci.*, 30: 211–219.
- Carey, R.J., Depalma, G., Damianopoulos, E., Muller, C.P. and Huston, J.P. (2004) The 5-HT_{1A} receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. *Psychopharmacology*, 177: 46–54.
- Carlsson, T., Carta, M., Winkler, C., Bjorklund, A. and Kirik, D. (2007) Serotonin neuron transplants exacerbate L-DOPA-induced dyskinesias in a rat model of Parkinson's disease. *J. Neurosci.*, 27: 8011–8022.
- Carta, M., Carlsson, T., Kirik, D. and Bjorklund, A. (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in Parkinsonian rats. *Brain*, 130: 1819–1833.
- Casasnovas, J.M., Hervas, I. and Artigas, F. (1999) Postsynaptic 5-HT_{1A} receptors control 5-HT release in the rat medial prefrontal cortex. *NeuroReport*, 10: 1441–1445.

- Ceci, A., Baschiroto, A. and Borsini, F. (1994) The inhibitory effect of 8-OH-DPAT on the firing activity of dorsal raphe serotonergic neurons in rats is attenuated by lesion of the frontal cortex. *Neuropharmacology*, 33: 709–713.
- Cenci, M.A., Lee, C.S. and Bjorklund, A. (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *Eur. J. Neurosci.*, 10: 2694–2706.
- Chalmers, D.T. and Watson, S.J. (1991) Comparative anatomical distribution of 5-HT_{1A} receptor mRNA and 5-HT_{1A} binding in rat brain — a combined in situ hybridisation/in vitro receptor autoradiographic study. *Brain Res.*, 561: 51–60.
- Chase, T.N. (1998) Levodopa therapy: consequences of the nonphysiologic replacement of dopamine. *Neurology*, 50: S17–S25.
- Cragg, S.J. and Rice, M.E. (2004) Dancing past the DAT at a DA synapse. *Trends Neurosci.*, 27: 270–277.
- Crosby, N.J., Deane, K.H. and Clarke, C.E. (2003) Amantadine for dyskinesia in Parkinson's disease. *Cochrane Database Syst. Rev.*, 2: p. CD003467.
- de la Fuente-Fernandez, R., Schulzer, M., Mak, E., Calne, D.B. and Stoessl, A.J. (2004) Presynaptic mechanisms of motor fluctuations in Parkinson's disease: a probabilistic model. *Brain*, 127: 888–899.
- Dunah, A.W., Sirianni, A.C., Fienberg, A.A., Bastia, E., Schwarzschild, M.A. and Standaert, D.G. (2004) Dopamine D1-dependent trafficking of striatal *N*-methyl-D-aspartate glutamate receptors requires Fyn protein tyrosine kinase but not DARPP-32. *Mol. Pharmacol.*, 65: 121–129.
- Dunah, A.W. and Standaert, D.G. (2001) Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *J. Neurosci.*, 21: 5546–5558.
- Dunah, A.W., Wang, Y., Yasuda, R.P., Kameyama, K., Hagan, R.L., Wolfe, B.B. and Standaert, D.G. (2000) Alterations in subunit expression, composition, and phosphorylation of striatal *N*-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. *Mol. Pharmacol.*, 57: 342–352.
- Eskow, K.L., Gupta, V., Alam, S., Park, J.Y. and Bishop, C. (2007) The partial 5-HT_{1A} agonist buspirone reduces the expression and development of L-DOPA-induced dyskinesia in rats and improves L-DOPA efficacy. *Pharmacol. Biochem. Behav.*, 87: 306–314.
- Everett, G.M. and Borcharding, J.W. (1970) L-DOPA: effect on concentrations of dopamine, norepinephrine, and serotonin in brains of mice. *Science*, 168: 847–850.
- Fahn, S. (2003) Description of Parkinson's disease as a clinical syndrome. *Ann. N.Y. Acad. Sci.*, 991: 1–14.
- Fiorentini, C., Rizzetti, M.C., Busi, C., Bontempi, S., Collo, G., Spano, P. and Missale, C. (2006) Loss of synaptic D1 dopamine/*N*-methyl-D-aspartate glutamate receptor complexes in L-DOPA-induced dyskinesia in the rat. *Mol. Pharmacol.*, 69: 805–812.
- Gardoni, F., Picconi, B., Ghiglieri, V., Polli, F., Bagetta, V., Bernardi, G., Cattabeni, F., Di Luca, M. and Calabresi, P. (2006) A critical interaction between NR2B and MAGUK in L-DOPA induced dyskinesia. *J. Neurosci.*, 26: 2914–2922.
- Goetz, C.G., Damier, P., Hicking, C., Laska, E., Muller, T., Olanow, C.W., Rascol, O. and Russ, H. (2007) Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. *Mov. Disord.*, 22: 179–186.
- Goodwin, G.M., De Souza, R.J., Wood, A.J. and Green, A.R. (1986) The enhancement by lithium of the 5-HT_{1A} mediated serotonin syndrome produced by 8-OH-DPAT in the rat: evidence for a post-synaptic mechanism. *Psychopharmacology*, 90: 488–493.
- Guigoni, C., Doudnikoff, E., Li, Q., Bloch, B. and Bezard, E. (2007) Altered D(1) dopamine receptor trafficking in Parkinsonian and dyskinetic non-human primates. *Neurobiol. Dis.*, 26: 452–463.
- Hadj Tahar, A., Gregoire, L., Darre, A., Belanger, N., Meltzer, L. and Bedard, P.J. (2004) Effect of a selective glutamate antagonist on L-DOPA-induced dyskinesias in drug-naïve Parkinsonian monkeys. *Neurobiol. Dis.*, 15: 171–176.
- Hajos, M., Hajos-Korcsok, E. and Sharp, T. (1999) Role of the medial prefrontal cortex in 5-HT_{1A} receptor-induced inhibition of 5-HT neuronal activity in the rat. *Br. J. Pharmacol.*, 126: 1741–1750.
- Hallett, P.J., Dunah, A.W., Ravenscroft, P., Zhou, S., Bezard, E., Crossman, A.R., Brotchie, J.M. and Standaert, D.G. (2005) Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. *Neuropharmacology*, 48: 503–516.
- Hollister, A.S., Breese, G.R. and Mueller, R.A. (1979) Role of monoamine neural systems in l-dihydroxyphenylalanine-stimulated activity. *J. Pharmacol. Exp. Ther.*, 208: 37–43.
- Hoyer, D., Hannon, J.P. and Martin, G.R. (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol. Biochem. Behav.*, 71: 533–554.
- Iravani, M.M., Tavarani-Binazir, K., Chu, W.B., Jackson, M.J. and Jenner, P. (2006) In MPTP treated primates, the selective 5-HT_{1A} agonist (*R*)-(+)-8-hydroxy-DPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. *J. Pharmacol. Exp. Ther.*, 319: 1225–1234.
- Jackson, M.J., Al-Barghouthy, G., Pearce, R.K., Smith, L., Hagan, J.J. and Jenner, P. (2004) Effect of 5-HT_{1B/D} receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. *Pharmacol. Biochem. Behav.*, 79: 391–400.
- Kannari, K., Tanaka, H., Maeda, T., Tomiyama, M., Suda, T. and Matsunaga, M. (2000) Reserpine pretreatment prevents increases in extracellular striatal dopamine following L-DOPA administration in rats with nigrostriatal denervation. *J. Neurochem.*, 74: 263–269.
- Kannari, K., Yamato, H., Shen, H., Tomiyama, M., Suda, T. and Matsunaga, M. (2001) Activation of 5-HT_{1A} but not 5-HT_{1B} receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. *J. Neurochem.*, 76: 1346–1353.
- Knobelman, D.A., Kung, H.F. and Lucki, I. (2000) Regulation of extracellular concentrations of 5-hydroxytryptamine

- (5-HT) in mouse striatum by 5-HT(1A) and 5-HT(1B) receptors. *J. Pharmacol. Exp. Ther.*, 292: 1111–1117.
- Langston, J.W. and Ballard, P. (1984) Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): implications for treatment and the pathogenesis of Parkinson's disease. *Can. J. Neurol. Sci.*, 11: 160–165.
- Langston, J.W., Forno, L.S., Rebert, C.S. and Irwin, I. (1984a) Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in the squirrel monkey. *Brain Res.*, 292: 390–394.
- Langston, J.W., Irwin, I., Langston, E.B. and Forno, L.S. (1984b) 1-Methyl-4-phenylpyridinium ion (MPP⁺): identification of a metabolite of MPTP, a toxin selective to the substantia nigra. *Neurosci. Lett.*, 48: 87–92.
- Langston, J.W., Langston, E.B. and Irwin, I. (1984c) MPTP-induced Parkinsonism in human and non-human primates — clinical and experimental aspects. *Acta Neurol. Scand. Suppl.*, 100: 49–54.
- Lavoie, B. and Parent, A. (1990) Immunohistochemical study of the serotonergic innervation of the basal ganglia in the squirrel monkey. *J. Comp. Neurol.*, 299: 1–16.
- Lin, J.Y., Dubey, R., Funk, G.D. and Lipski, J. (2003) Receptor subtype-specific modulation by dopamine of glutamatergic responses in striatal medium spiny neurons. *Brain Res.*, 959: 251–262.
- Lopez, A., Munoz, A., Guerra, M.J. and Labandeira-Garcia, J.L. (2001) Mechanisms of the effects of exogenous levodopa on the dopamine-denervated striatum. *Neuroscience*, 103: 639–651.
- Lopez-Real, A., Rodriguez-Pallares, J., Guerra, M.J. and Labandeira-Garcia, J.L. (2003) Localization and functional significance of striatal neurons immunoreactive to aromatic L-amino acid decarboxylase or tyrosine hydroxylase in rat Parkinsonian models. *Brain Res.*, 969: 135–146.
- Luginger, E., Wenning, G.K., Bosch, S. and Poewe, W. (2000) Beneficial effects of amantadine on L-DOPA-induced dyskinesias in Parkinson's disease. *Mov. Disord.*, 15: 873–878.
- Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N. and Cenci, M.A. (2002) Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur. J. Neurosci.*, 15: 120–132.
- Lundblad, M., Usiello, A., Carta, M., Hakansson, K., Fisone, G. and Cenci, M.A. (2005) Pharmacological validation of a mouse model of L-DOPA-induced dyskinesia. *Exp. Neurol.*, 194: 66–75.
- Maeda, T., Nagata, K., Yoshida, Y. and Kannari, K. (2005) Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered L-DOPA. *Brain Res.*, 1046: 230–233.
- Mayeux, R. (2003) Epidemiology of neurodegeneration. *Annu. Rev. Neurosci.*, 26: 81–104.
- Melamed, E., Hefti, F., Pettibone, D.J., Liebman, J. and Wurtman, R.J. (1981) Aromatic L-amino acid decarboxylase in rat corpus striatum: implications for action of L-DOPA in Parkinsonism. *Neurology*, 31: 651–655.
- Mignon, L.J. and Wolf, W.A. (2005) 8-hydroxy-2-(di-*n*-propylamino)tetralin reduces striatal glutamate in an animal model of Parkinson's disease. *NeuroReport*, 16: 699–703.
- Morissette, M., Dridi, M., Calon, F., Tahar, A.H., Meltzer, L.T., Bedard, P.J. and Di Paolo, T. (2006a) Prevention of levodopa-induced dyskinesias by a selective NR1A/2B N-methyl-D-aspartate receptor antagonist in Parkinsonian monkeys: implication of preproenkephalin. *Mov. Disord.*, 21: 9–17.
- Morissette, M., Dridi, M., Calon, F., Tahar, A.H., Meltzer, L.T., Bedard, P.J. and Di Paolo, T. (2006b) Prevention of dyskinesia by an NMDA receptor antagonist in MPTP monkeys: effect on adenosine A2A receptors. *Synapse*, 60: 239–250.
- Mura, A., Jackson, D., Manley, M.S., Young, S.J. and Groves, P.M. (1995) Aromatic L-amino acid decarboxylase immunoreactive cells in the rat striatum: a possible site for the conversion of exogenous L-DOPA to dopamine. *Brain Res.*, 704: 51–60.
- Ng, K.Y., Colburn, R.W. and Kopin, I.J. (1971) Effects of L-DOPA on efflux of cerebral monoamines from synaptosomes. *Nature*, 230: 331–332.
- Ng, K.Y., Chase, T.N., Colburn, R.W. and Kopin, I.J. (1970) L-DOPA-induced release of cerebral monoamines. *Science*, 170: 76–77.
- Nicholson, S.L. and Brotchie, J.M. (2002) 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease — opportunities for novel therapeutics to reduce the problems of levodopa therapy. *Eur. J. Neurol.*, 9(Suppl 3): 1–6.
- Nyholm, D. and Aquilonius, S.M. (2004) Levodopa infusion therapy in Parkinson disease: state of the art in 2004. *Clin. Neuropharmacol.*, 27: 245–256.
- Obeso, J.A., Olanow, C.W. and Nutt, J.G. (2000) Levodopa motor complications in Parkinson's disease. *Trends Neurosci.*, 23: S2–S7.
- Olanow, C.W., Damier, P., Goetz, C.G., Mueller, T., Nutt, J., Rascol, O., Serbanescu, A., Deckers, F. and Russ, H. (2004) Multicenter, open-label, trial of sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). *Clin. Neuropharmacol.*, 27: 58–62.
- Olanow, C.W. and Obeso, J.A. (2000) Pulsatile stimulation of dopamine receptors and levodopa-induced motor complications in Parkinson's disease: implications for the early use of COMT inhibitors. *Neurology*, 55: S72–S77. discussion S78–S81.
- Olanow, W., Schapira, A.H. and Rascol, O. (2000) Continuous dopamine-receptor stimulation in early Parkinson's disease. *Trends Neurosci.*, 23: S117–S126.
- Peter, D., Jimenez, J., Liu, Y., Kim, J. and Edwards, R.H. (1994) The chromaffin granule and synaptic vesicle amine transporters differ in substrate recognition and sensitivity to inhibitors. *J. Biol. Chem.*, 269: 7231–7237.
- Picconi, B., Centonze, D., Hakansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A. and Calabresi, P. (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat. Neurosci.*, 6: 501–506.

- Picconi, B., Paille, V., Ghiglieri, V., Bagetta, V., Barone, I., Lindgren, H.S., Bernardi, G., Angela Cenci, M. and Calabresi, P. (2007) L-DOPA dosage is critically involved in dyskinesia via loss of synaptic depotentiation. *Neurobiol. Dis.*, 29(2): 327–335.
- Pisani, A., Centonze, D., Bernardi, G. and Calabresi, P. (2005) Striatal synaptic plasticity: implications for motor learning and Parkinson's disease. *Mov. Disord.*, 20: 395–402.
- Riad, M., Garcia, S., Watkins, K.C., Jodoin, N., Doucet, E., Langlois, X., el Mestikawy, S., Hamon, M. and Descarries, L. (2000) Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J. Comp. Neurol.*, 417: 181–194.
- Robelet, S., Melon, C., Guillet, B., Salin, P. and Kerkerian-Le Goff, L. (2004) Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a rat model of Parkinson's disease. *Eur. J. Neurosci.*, 20: 1255–1266.
- Santini, E., Valjent, E., Uziel, A., Carta, M., Borgkvist, A., Girault, J.A., Herve, D., Greengard, P. and Fisone, G. (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. *J. Neurosci.*, 27: 6995–7005.
- Sari, Y., Miquel, M.C., Brisorgueil, M.J., Ruiz, G., Doucet, E., Hamon, M. and Verge, D. (1999) Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience*, 88: 899–915.
- Smith, L.M. and Peroutka, S.J. (1986) Differential effects of 5-hydroxytryptamine1a selective drugs on the 5-HT behavioral syndrome. *Pharmacol. Biochem. Behav.*, 24: 1513–1519.
- Sprouse, J.S. and Aghajanian, G.K. (1987) Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. *Synapse*, 1: 3–9.
- Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T. and Matsunaga, M. (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *NeuroReport*, 10: 631–634.
- Venton, B.J., Zhang, H., Garriss, P.A., Phillips, P.E., Sulzer, D. and Wightman, R.M. (2003) Real-time decoding of dopamine concentration changes in the caudate-putamen during tonic and phasic firing. *J. Neurochem.*, 87: 1284–1295.
- Westin, J.E., Andersson, M., Lundblad, M. and Cenci, M.A. (2001) Persistent changes in striatal gene expression induced by long-term L-DOPA treatment in a rat model of Parkinson's disease. *Eur. J. Neurosci.*, 14: 1171–1176.
- Yamada, J., Sugimoto, Y. and Horisaka, K. (1988) The behavioural effects of 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) in mice. *Eur. J. Pharmacol.*, 154: 299–304.

CHAPTER 23

Parkinson's disease — opportunities for novel therapeutics to reduce the problems of levodopa therapy

Susan H. Fox^{1,*}, Rosalind Chuang¹ and Jonathan M. Brotchie²

¹*Movement Disorders Clinic, University of Toronto, Toronto Western Hospital, Toronto, Ontario, Canada M5T 2S8*

²*Toronto Western Research Institute, Toronto, Ontario, Canada M5T 2S8*

Abstract: Long-term treatment for Parkinson's disease (PD) with the dopamine-precursor levodopa (L-DOPA) results in the development of motor fluctuations, including involuntary movements, termed L-DOPA-induced dyskinesia (LID). Currently, effective treatments for LID are limited. The neurodegenerative processes underlying PD result in loss of serotonin (5-HT) input from the dorsal raphe nucleus (DRN) to the striatum, but to a lesser extent than loss of dopamine input. L-DOPA may be converted to dopamine in remaining serotonergic neurons and the non-physiological release of dopamine may lead to abnormal dopamine receptor stimulation in the striatopallidal pathways and result in the generation of LID. Suppressing the activity of these 5-HT inputs to the striatum via presynaptic 5-HT_{1A} agonists may reduce LID. However, to date, studies with 5-HT_{1A} agonists have suggested a reduction in LID, but with worsening PD disability. Postsynaptic 5-HT_{2A} and 5-HT_{2C} receptors in the striatum may modulate dopamine to reduce LID and the atypical antipsychotic, clozapine is effective at reducing LID without worsening PD. Alternatively, postsynaptic 5-HT_{1A}, presynaptic 5-HT_{1B/1D} receptors and 5-HT_{2C} receptors may modulate GABA and glutamate release within other basal ganglia nuclei to reduce LID. Thus, 5-HT ligands can modulate basal ganglia function and hence motor function through several receptor subtypes and locations, with potential therapeutic benefit to the motor complications induced by long-term L-DOPA therapy in PD. Future studies are needed to develop 5-HT selective drugs that can reduce LID without affecting the anti-parkinsonian action of L-DOPA.

Keywords: Parkinson's disease; L-DOPA-induced dyskinesia; serotonin

Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterised by slowness of movement (bradykinesia), muscle rigidity, a 4–6 Hz

resting tremor and postural instability. The predominant pathology of PD is degeneration of dopaminergic neurons within the midbrain, particularly substantia nigra pars compacta. However, increasingly it is appreciated that other pathology exists in non-dopaminergic regions including the serotonergic dorsal raphe nucleus (DRN) (Halliday et al., 1990). The principal treatment for PD remains reliant on dopamine replacement therapy,

*Corresponding author. Tel.: +1 416 603 5383/6422;
Fax: +1 416 603 5004; E-mail: sfox@uhnresearch.ca

particularly as the dopamine precursor, L-DOPA. However after chronic use, most patients begin to experience a range of L-DOPA-induced motor complications. These include shortening of the duration of action of individual doses of L-DOPA, termed 'wearing-off', which can be predictable or sudden or unpredictable. In addition, patients may experience no effect of a dose of L-DOPA, or reduced benefit to L-DOPA, with delayed on response or dose failure. Patients may also cycle between on and off states, termed 'on-off' fluctuations (Lang and Lozano, 1998).

In addition, patients experience involuntary, hyperkinetic, abnormal movements termed L-DOPA-induced dyskinesia (LID). These involuntary movements include chorea, dystonia or ballism that may affect the head, trunk or limbs, and tend to involve the side of the body or the limb most affected by parkinsonism. Motor activity or emotional stress can exacerbate LID. Dyskinesia can be seen in any stage of the L-DOPA dose, often described temporally as 'peak dose', 'diphasic' (onset and end of dose) and 'off-period' dyskinesia. When dyskinesia appears during the peak plasma concentration of L-DOPA, the movements tend to be choreic whereas off-period or diphasic dyskinesia is predominantly dystonic. LID is a common problem and develops in 30–80% of PD patients with incidence varying by age of onset and treatment duration. The incidence in patients who develop PD after age 70, after 5 years of treatment, is 16%, whereas younger onset PD patients (between 40 and 59 years) have increased risk at 50% (Kumar et al., 2005; Van Gerpen et al., 2006). In addition to social embarrassment, the movements contribute to overall disability and may be more debilitating than the disease itself (Dodel et al., 2001).

Neural mechanisms underlying LID

The neural mechanisms underlying LID result from a combination of pre- and postsynaptic changes in the nigrostriatal dopaminergic system and possibly other dopamine pathways, e.g. the nigropallidal pathway (Mouradian et al., 1988; Obeso et al., 2000; Bezard et al., 2001). [To date, the proposed pathophysiology of LID probably relates to

peak-dose dyskinesia as animal models (in particular 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primate model of PD) exhibit peak-dose dyskinesia and rarely diphasic dystonia (Brotchie, 2000).] Thus, with disease progression, there is loss of nigrostriatal dopaminergic terminals, resulting in altered central pharmacokinetics of L-DOPA (presynaptic changes); combined with altered peripheral pharmacokinetics of L-DOPA with erratic absorption and, combined with the duration and dose of L-DOPA therapy, results in abnormal intra-synaptic dopamine concentration with loss of normal constant stimulation of postsynaptic dopamine receptors.

These central pharmacodynamic or postsynaptic changes within the striatum are thought to cause an increased activity in the direct, dopamine D₁ receptor-mediated striatal output pathway, resulting in a net reduction in activity from the basal ganglia output regions, i.e. medial segment of the globus pallidus (GPM) and the substantia nigra pars reticulata (SNr) to thalamocortical pathways (Crossman, 1990; Obeso et al., 2000). As a consequence of altered rate and pattern of activity in these basal ganglia connections abnormal involuntary movements, LID occurs. Multiple neurotransmitter and neuromodulatory systems are potentially involved in the neural mechanisms underlying the development of LID (Brotchie, 2005). Peak-dose LID will respond to a reduction in dopamine replacement therapy; however, with a resultant increase in parkinsonian disability. Thus, potential therapeutic options for LID include non-dopaminergic treatments that allow a reduction in dyskinesia without affecting motor function. To date, the most effective non-dopaminergic therapy for LID involves the NMDA receptor antagonist, amantadine (Verhagen Metman et al., 1998), but this has restrictions due to significant adverse effects on cognition, including sedation and development of psychosis. New therapeutic targets are therefore required.

Methods for evaluating dyskinesia in PD

Animal models of PD commonly used to evaluate potential therapies for LID and other motor

complications of L-DOPA therapy include the unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model and the MPTP-lesioned primate model of PD. The 6-OHDA-lesioned rat, following long-term treatment with L-DOPA, develops a progressive increase in rotational responses to L-DOPA that are contra-lateral to the side of the dopaminergic lesion, often with a shortening of duration of responses; these behavioural changes have pharmacological and biochemical correlates to LID in PD patients (Henry et al., 1998). More recently, involuntary movements of the limbs and orofacial muscles in these animals have been proposed to model dyskinesia and can be measured using an abnormal involuntary movements rating scale (AIMS) (Lundblad et al., 2002). However, the 6-OHDA-lesioned rat may have limited predictive value as a model for LID in PD as the phenomenology of dyskinesia is different to that seen in the patient and an effect on parkinsonian disability cannot be adequately assessed in the rat. The MPTP-lesioned primate model of PD is widely acknowledged as a validated model of the motor symptoms of PD (Jenner, 2003). Thus, MPTP-lesioned primates exhibit bradykinesia, postural instability and a reduced range of movement. Moreover, following long-term L-DOPA therapy MPTP-lesioned animals develop involuntary movements that are identical to chorea and dystonia seen in PD patients (Pearce et al., 1995; Henry et al., 2001). Parkinsonian disability and dyskinesia can be rated using a range of rating scales, similar to those used in PD patients (see below) (reviewed in Fox et al., 2006).

Clinical rating scales have been developed to measure LID in PD patients. The two most frequently used in clinical studies are the AIMS and the RDRS (Rush Dyskinesia Rating Scale). AIMS is a purely objective scale that rates dyskinesia in seven different body parts at rest and includes three areas of global assessment of overall severity, extent of disability and patient awareness of dyskinesia (Guy, 1976). One main limitation is that it does not measure dyskinesia in actions pertinent to activities of daily living (ADL) and was originally designed for the evaluation of tardive dyskinesia. The RDRS (Goetz et al., 1994) allows for an assessment of functional disability

during direct observed tasks, such as drinking from a cup or buttoning, but again does not correlate to patient's perception of disability.

The Core Assessment Program for Surgical Interventional Therapies in PD (CAPSIT-PD) scale (Widner and Defer, 1999) uses a combination of home diary and direct physician rating of dyskinesia severity during 'on' and 'off' states to determine impact on function. The limitation of this scale is that the motor tasks (finger tapping, supination/pronation, arising from chair, etc.) lack the full range of movements necessary for normal daily activities. A combination of CAPSIT and RDRS is the Lang-Fahn ADL scale, which accounts for function during ADL tasks of writing, eating, dressing, hygiene and walking, with home-based diaries and physician-observed rating (Parkinson Study Group, 2001). The Unified Parkinson's disease rating scale (UPDRS), the most widely used rating scale for assessing PD, also has a component for measuring LID. However, this is a subjective measure based on historical recall and measures the amount of dyskinesia and the level of perceived disability. Thus to date, all scales used in evaluating LID in PD have limitations.

5-HT and the basal ganglia in PD

5-HT projections from the DRN innervate all components of the basal ganglia circuitry. In particular, the striatum and output regions of the basal ganglia (SNr and GPM) receive a dense serotonergic (5-HT) input (Lavoie and Parent, 1990). Recent pathological studies have suggested that while there is loss of dorsal raphe neurons in PD, there is a relative preservation of serotonergic input to the basal ganglia in PD compared to loss of dopamine (30–60% compared to >90% loss, respectively) particularly to the putamen; there are no differences in serotonin metabolites between PD patients with or without dyskinesia (Kish et al., 2008). Early studies suggested that the relatively intact serotonergic input to the basal ganglia may help conversion of L-DOPA to dopamine, as a false neurotransmitter (Ng et al., 1970, 1971) and that dopamine is released from 5-HT neurons

(Tanaka et al., 1999). However, because dopamine is released in a non-physiological way from serotonergic terminals, then this may be partly responsible for LID (Carta et al., 2007). In the 6-OHDA-lesioned rat model of PD, dopamine neuron-rich foetal grafts have been shown to induce a functional recovery, with a reduction in LID, however, in contrast, when animals receive serotonin-rich grafts, with few dopamine neurons, there was a progressive worsening of LID over time and no functional improvement, suggesting a role of 5-HT in the generation of LID (Carlsson et al., 2007).

5-HT has been shown to modulate dopaminergic neurotransmission in the striatum as well as GABA and glutamate neurotransmission in the output regions of the basal ganglia via a number of 5-HT receptors (see earlier chapters), suggesting a role for 5-HT in movement control (Nicholson and Brotchie, 2002). Of these, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors are discussed in Table 1.

5-HT_{1A} receptors

5-HT_{1A} receptors are involved in control of 5-HT release via autoreceptors in the DRN as well as potential modulation of dopamine, GABA and glutamate neurotransmission via postsynaptic receptors within the basal ganglia (see earlier chapters). 5-HT_{1A} receptors are up-regulated in the striatum of untreated MPTP-primate, particularly the striosomes of the caudal putamen (Frechilla et al., 2001). However, changes in 5-HT_{1A} receptors after long-term L-DOPA treatment and the development of LID are unknown. Postmortem studies, in PD patients after long-term L-DOPA (although specific clinical details regarding presence of LID are not stated) have shown either no change (Waeber and Palacios, 1989) or increased 5-HT_{1A} receptors in the neocortex, compared to age-matched controls (Chen et al., 1998).

Preclinical studies of 5-HT_{1A} ligands in animal models of LID

Behavioural studies have shown that systemic administration of R-(+)-8-OH-DPAT or the

partial 5-HT_{1A} agonist, buspirone, can reduce dyskinesia in the L-DOPA-treated 6-OHDA-lesioned rat model of PD (Carta et al., 2007; Dupre et al., 2007; Eskow et al., 2007). The non-selective serotonergic agent, 'ecstasy' (3,4-methylenedioxymethamphetamine, MDMA) has also been shown to reduce LID in the 6-OHDA-lesioned rat; an effect that is blocked by pretreatment with the 5-HT_{1A} antagonist WAY 100635 (Bishop et al., 2006). In addition, co-administration of R-(+)-8-OH-DPAT with L-DOPA has been shown to prevent behavioural sensitisation to L-DOPA, thus suggesting 5-HT_{1A} agonists may also prevent the development of LID, as well as reduce established LID (Tomiya et al., 2005).

In the MPTP-lesioned model of LID, the 5-HT_{1A} receptor agonist sarizotan alone had no effect on parkinsonian severity or on the anti-parkinsonian response to L-DOPA, but reduced L-DOPA-induced choreiform dyskinesia by $91 \pm 5.9\%$. The effects of sarizotan were blocked by the selective 5-HT_{1A} antagonist WAY 100635, suggesting the responses were probably mediated at the 5-HT_{1A} receptor (Bibbiani et al., 2001). However, sarizotan has actions at non-5-HT receptors and is also a dopamine D₂/D₃ receptor antagonist (Bartoszyk et al., 2004). MDMA has also been shown to reduce LID in the MPTP-lesioned primate with no detrimental effect on parkinsonism (Iravani et al., 2003). The effects were blocked by the selective serotonin reuptake inhibitor (SSRI), fluvoxamine and also partially by 5-HT_{1A} antagonist suggesting a possible role for 5-HT_{1A} receptors. The selective 5-HT_{1A} agonist, R-(+)-8-OH-DPAT, also reduced LID by 50% in MPTP-lesioned primates. However, this action was accompanied by a worsening of parkinsonian motor scores (Iravani et al., 2006). In addition, some animals were noted to exhibit postural abnormalities with shuffling and head-down postures similar to the serotonin syndrome seen in rodents.

The mechanism of action of 5-HT_{1A} agonists on reducing LID may be via presynaptic 5-HT_{1A} receptors in the DRN. As noted above, L-DOPA can be converted to 5-HT in serotonergic terminals and thus 5-HT_{1A} agonists, by reducing serotonergic activation, may reduce dopamine release in the

Table 1. 5-HT ligands in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease

5-HT receptor action	Drug	6-OHDA-lesioned rat model of LID	MPTP-lesioned primate model of LID	Clinical trials in PD patients with LID
5-HT _{1A} agonist	<i>R</i> -(+)-8-OH DPAT	Reduced AIMS and CL rotations in established dyskinesia induced by D ₁ , D ₂ agonists and L-DOPA (0.2–2.0 mg/kg) (Dupre et al., 2007). Reduced development of L-DOPA-induced CL rotations (Tomiya et al., 2005). Reduced AIMS in established LID (partial 6-OHDA lesion). No effect on mixed D ₁ /D ₂ agonist, apomorphine-induced AIMS (0.05–0.2 mg/kg) (Carta et al., 2007)	Reduced choreiform dyskinesia induced by L-DOPA and D ₂ /D ₃ agonist, pramipexole. Worsened parkinsonian motor scores (0.1–1.0 mg/kg) (Iravani et al., 2006)	
	Buspirone	Reduced AIMS and forepaw adjusting steps in established LID and prevented onset in de novo (0.25, 2.5 mg/kg) (Eskow et al., 2007). Reduced AIMS but worsened rotorod motor testing (1–4 mg/kg) (Dekundy et al., 2007)		RCT (20 mg/d) reduced LID using AIMS, no effect on PD scores (<i>n</i> = 10) (Bonifati et al., 1994). Tansospirone (15–60 mg/day) reduced LID with worsening PD scores in 50% (<i>n</i> = 10) (Kannari et al., 2002)
	Sarizotan	Reduced progressive L-DOPA-induced shortening of CL rotations (analogous to wearing-off) (2.5 mg/kg) (Bibbiani et al., 2001)	Reduced LID; no adverse effect on parkinsonian motor scores (Bibbiani et al., 2001)	Open label (mean dose 8.9 mg/d) reduced LID (AIMS and RDRS; worsened PD (<i>n</i> = 64) (Olanow et al., 2004). Phase IIa RCT (5 mg b.i.d.) reduced LID (AIMS) without affecting PD motor scores with optimal dose of i.v. L-DOPA (<i>n</i> = 18) (Bara-Jimenez et al., 2005). Phase IIb RCT (2, 4 and 10 mg/d). Reduced LID using AIMS, UPDRS and diary; 4 and 10 mg/d also reduced PD scores (<i>n</i> = 398) (Goetz et al., 2007). Phase III RCT, PADDY-1 (2 mg/d) (<i>n</i> = 504) and PADDY-2 (2 mg/d) (<i>n</i> = 403) using UPDRS and diary. No effect on LID compared to placebo; no effect on PD motor scores compared to placebo (preliminary reported in Rascol et al., 2006; Müller et al., 2006) Large placebo effect reported (Goetz et al., 2008)

TABLE 1. (Continued)

5-HT receptor action	Drug	6-OHDA-lesioned rat model of LID	MPTP-lesioned primate model of LID	Clinical trials in PD patients with LID
Mixed 5-HT _{1A} agonist, 5-HT ₂ antagonist, (alpha ₂ antagonist)	Mirtazapine			Open label (30 mg/d) reduced LID using AIMS; no worsening of PD scores (<i>n</i> = 20) (Meco et al., 2003)
	SKF 99101		Reduced LID, but worsened parkinsonian disability (Jackson et al., 2004)	
	CP-94253	Reduced AIMS (partial 6-OHDA-lesioned) (2.5 mg/kg) (Carta et al., 2007)		
5-HT _{1B} agonist				
Mixed 5-HT _{1A} and 1B agonist	MDMA	Reduced AIMS and CL rotations (0.25 or 2.5 mg/kg) (Bishop et al., 2006)	Reduced LID (chorea > dystonia) and pramipexole-induced dyskinesia; no adverse effect on parkinsonian motor scores (3, 12 mg/kg) ((Irvani et al., 2003)	
5-HT _{2A/2C} antagonist	MDL 100907	Reduces dopamine D ₁ but not D ₂ - or L-DOPA-induced CL rotations (Taylor et al., 2006)		
	Methysergide		Reduced LID; but worsened parkinsonian disability (Gomez-Mancilla and Bedard, 1993)	
	Quetiapine		Reduced LID without affecting parkinsonian disability (4.0 mg/kg) (Oh et al., 2002), Reduced LID without affecting parkinsonian disability (0.5–4.5 mg/kg) (Visanji et al., 2006)	RCT (25 mg/d) no effect of LID or PD scores (<i>n</i> = 9) (Katzenschlager et al., 2004)

Clozapine	5-HT _{2A} partial agonist	ACP 103	Reduced LID with worsening parkinsonian disability at high dose (0.1–1.0 mg/kg) (Grondin et al., 1999). Reduced LID without affecting parkinsonian disability (0.1–1.0 mg/kg) (Visanji et al., 2006)	RCT (mean dose 39.4 mg/d); reduced LID using RDRS, no effect on parkinsonian motor scores (<i>n</i> = 50) (Durif et al., 2004)
		J-18	Reduced LID without affecting parkinsonian disability (0.1–0.3 mg/kg) (Hadj Tahar et al., 2000)	
		5-MDOT	Reduced LID but worsened PD motor scores (Gomez-Mancilla and Bedard, 1993)	RCT reduced LID no effect on (Roberts, 2006) (<i>n</i> = 12)
		SSRI	Reduced L-DOPA-induced CL rotations (Henry et al., 1998)	
Fluoxetine	Fluoxetine	Fluvoxamine	No effect on LID (Iravani et al., 2003)	Open label (20 mg/d) reduced apomorphine-induced dyskinesia, no effect on PD (<i>n</i> = 7) (Durif et al., 1995)
		Fluoxetine		RCT cross over (20 mg/d). No effect on LID (<i>n</i> = 15) (Chung et al., 2005)
		Paroxetine		

Key: AIMS, abnormal involuntary movement rating scale; CL, contra lateral rotations; LID, levodopa (L-DOPA)-induced dyskinesia; PD, Parkinson's disease; RCT, randomised controlled trial; RDRS, rush dyskinesia rating scale; SSRI, serotonin selective reuptake inhibitor; UPDRS, unified Parkinson's disease rating scale.

striatum (Santiago et al., 1998). Indeed, studies in the 6-OHDA-lesioned rat model of PD have shown that administration of the 5-HT_{1A} agonist *R*-(+)-8-OH-DPAT with L-DOPA reduces extracellular dopamine levels (Kannari et al., 2001). Thus, 5-HT_{1A} agonists may reduce LID by reducing dopamine release within the striatum. However, the worsening of parkinsonism seen with some 5-HT_{1A} agonists may also relate to this reduction in dopamine release in the striatum as a result of an effect on L-DOPA metabolism. However, this cannot provide a complete explanation as identical effects on LID and worsening parkinsonism can occur when *R*-(+)-8-OH-DPAT is administered with the direct postsynaptic dopamine D₂/D₃ agonist, pramipexole (Iravani et al., 2006).

An alternative site of action in reducing LID may be 5-HT_{1A} receptor stimulation within the striatum to reduce dopamine, 5-HT release and reduce glutamatergic activity; the latter is thought to underlie the generation of LID (Chase, 1998; Brotchie, 2000). Thus, intra-cortical sarizotan has been shown to reduce cortical and striatal glutamate levels in rodents, an effect blocked by selective 5-HT_{1A} antagonists (Antonelli et al., 2005). Another potential mechanism is via 5-HT_{1A} receptors within the globus pallidus. In normal awake monkeys using electrical recordings, 5-HT, acting via 5-HT_{1A} receptors, also suppresses pallidal bursting activity via glutamatergic mechanisms (Kita et al., 2007), although the effects occur within both pallidal segments (GPM and alateral lobe of globus pallidus (GPI)) and thus depending on site of action, could have potential to both reduce LID and worsen PD motor function.

Clinical studies of 5-HT_{1A} agonists in LID

The 5-HT_{1A} agonist, sarizotan has undergone clinical study in PD as a potential treatment for LID. In a Phase IIa study ($n = 18$), sarizotan 5 mg b.i.d., but not 2 mg b.i.d., reduced LID by 40% in advanced PD patients, without affecting the anti-parkinsonian action of optimal L-DOPA infusion (Bara-Jimenez et al., 2005). A larger open-label

Phase II study (SPLENDID) provided preliminary data on safety, tolerability and efficacy in 64 PD patients with moderate to severe dyskinesia (Olanow et al., 2004). The target dose for all patients was 10 mg b.i.d., but had to be reduced in 35 patients because of worsening parkinsonism, and 6 patients withdrew from the study altogether because of this adverse effect. Benefits were obtained with a mean daily dose of 8.9 mg. Of the 51 patients who completed the study, sarizotan reduced both AIMS and RDRS scores, as well as percent of on-time with dyskinesia. These data suggested that if appropriate dosing could be tolerated, sarizotan may be capable of providing anti-dyskinetic benefit. However, in 2007, Goetz et al. reported results from the first double-blind placebo-controlled trial of sarizotan (2, 4 and 10 mg/d) in 398 PD patients (Goetz et al., 2007). Dyskinesia was rated using patient diaries, AIMS scores and UPDRS. In the study, patients who received 2 mg daily of sarizotan noticed reduction in the UPDRS-II activities of daily living rating without worsening of UPDRS III parkinsonian motor subscores. However, in contrast to the earlier open-label study, sarizotan failed to reduce the amount of on-time without dyskinesia. Higher doses of 4 and 10 mg did not demonstrate anti-dyskinetic effects and were associated with increased 'off' times. Moreover, while patients had some measurable improvement compared to baseline, two additional double-blind placebo-controlled trials (PADDY-1 and PADDY-2) (to date reported in abstract only) (Table 1), in which placebo was compared to 2 mg of sarizotan, demonstrated that the improvement was no greater than placebo-treated patients (Goetz et al., 2008).

Other potential 5-HT_{1A} agonists that have undergone evaluation for LID in PD include a range of clinically available antidepressant drugs. In general, these studies have been in small numbers of patients and applicability to clinical practice remains unclear. In a double-blind placebo-controlled trial, the partial 5-HT_{1A} agonist, buspirone (20 mg/d) has been shown to reduce dyskinesia without worsening parkinsonian disability in 10 patients (Bonifati et al., 1994). In Japan, a buspirone-like drug, tandospirone citrate

(15–60 mg/day), was found to improve dyskinesia in 5 out of 10 PD patients; however, 50% also had worsening of their parkinsonism (Kannari et al., 2002). Mirtazapine is an antidepressant with multiple mechanisms of action that modulates serotonergic transmission with 5-HT_{1A} agonist and 5-HT₂ and 5-HT₃ antagonist actions as well as noradrenergic actions as an α -2 antagonist and anti-cholinergic functions (Stimmel et al., 1997). Mirtazapine has been found to reduce parkinsonian tremors (Gordon et al., 2002) and in 1999 reports of two cases in which mirtazapine improved dopamine induced dyskinesia (Pact and Giduz, 1999) led to an open-label study with 30 mg/d of mirtazapine in PD patients; 17 out of 20 patients completed the study while 3 dropped out because of visual hallucinations and confusion. In all 17 patients, the intensity of dyskinesia, based on AIMS scores, improved at 1, 3 and 6 months without worsening of their parkinsonism (Meco et al., 2003). To date, a randomised double-blind control trial has not been done. Moreover, at present it is unclear if the anti-dyskinetic effect is due to serotonergic or noradrenergic action. Other drugs with 5-HT_{1A} agonist properties in clinical development include SLV308. This agent is also a partial dopamine D₂/D₃ agonist (Glennon et al., 2006). Phase III studies are underway to determine efficacy in PD (Johnston and Brotchie, 2006).

5-HT_{1B} receptors

The localisation of 5-HT_{1B} receptors on the terminals of 5-HT neurons in the striatum and on GABAergic striatopallidal output neurons in the SNr and GP would suggest a role in control of basal ganglia function and thus potential to modulate symptoms of LID. Indeed, 5-HT_{1B} knockout mice exhibit hyperactivity suggesting a role in movement (Brunner et al., 1999). To date, limited studies have investigated changes in 5-HT_{1B} receptors in PD. One postmortem study demonstrated no change in 5-HT_{1B} receptors levels within the striatum and substantia nigra in six PD patients compared to age-matched controls (Castro et al., 1998).

Preclinical studies of 5-HT_{1B} agonists in animal models of LID

Studies in the 6-OHDA-lesioned rat have shown a reduction in LID with the 5-HT_{1B} agonist, CP-94253 (Carta et al., 2007). In the MPTP-primate, the non-selective 5-HT_{1B/1D} agonist SKF-99101, reduced LID but with a worsening of parkinsonian disability (Jackson et al., 2004). As noted above, the non-selective serotonergic drug MDMA can reduce LID in the MPTP-lesioned primate; an action that is blocked by 5-HT_{1B} antagonists (Iravani et al., 2006).

Several mechanisms may underlie the ability of 5-HT_{1B} agonists to reduce LID. Stimulation of 5-HT_{1B} receptors within the striatum can reduce 5-HT release (Knobelman et al., 2000), which may reduce L-DOPA-metabolism to dopamine, in serotonergic neurons and hence reduce dopamine release, and dyskinesia, in a similar way to 5-HT_{1A} agonist actions (Carta et al., 2007). In addition, stimulation of 5-HT_{1B} receptors in the SNr reduces GABA release (Stanford and Lacey, 1996). 5-HT activation of 5-HT_{1B} receptors also suppresses GABA release in the pallidum in electrical recording from normal awake monkeys following motor cortex stimulation with activation of the cortico–STN–pallidal glutamatergic pathway (Kita et al., 2007). Thus, depending on the site of action, 5-HT_{1B} receptor agonists may reduce dyskinesia by reduced GABA release in the output regions of the basal ganglia or reduce PD by reducing GABA release in the GPI, thus may be effective treatment for LID without worsening PD motor scores. Thus, 5-HT_{1B} agonists are plausible candidates for development as anti-dyskinetic agents. However, to date no clinical trials have been performed evaluating selective 5-HT_{1B/1D} agonists in LID.

5-HT_{2A} receptors

5-HT_{2A} receptors are widely distributed in the basal ganglia (see earlier chapters). There are limited studies on changes in 5-HT_{2A} receptor distribution and levels in PD or LID. In 6-OHDA-lesioned rats, there is an increase in 5-HT_{2A} mRNA within the striatum (Numan et al., 1995), which is reversed after L-DOPA treatment and the

development of LID (Zhang et al., 2007). This suggests that 5-HT_{2A} function is closely linked to changes in dopamine levels in the parkinsonian striatum. Pathological studies have shown an increase in 5-HT_{2A} receptors in the neocortex of PD but no correlation to severity or presence of LID was performed (Chen et al., 1998).

Preclinical studies of 5-HT_{2A} antagonists in animal models of LID

The selective 5-HT_{2A} receptor antagonist, M 100907 does not reduce L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model but reduces dyskinesia induced by the dopamine D₁ agonist SKF 82958 (Taylor et al., 2006). This has been suggested to be linked to 5-HT_{2A}-mediated normalisation in pre-protachykinin mRNA in the rostral striatum following dopamine depletion, with increased activation of the dopamine D₁-mediated direct striatopallidal pathway and resultant under-activity of basal ganglia outputs and dyskinesia (Bishop et al., 2003). The lack of effect on L-DOPA-induced dyskinesia, though, is unclear.

Studies in the MPTP-lesioned primate have shown that methysergide, a non-selective 5-HT₂ antagonist, can reduce LID, but with adverse effect on parkinsonism (Gomez-Mancilla and Bedard, 1993). The atypical neuroleptics, clozapine and quetiapine have 5-HT_{2A/2C} antagonist properties, as well as dopamine D₂ antagonist properties. Indeed, clozapine has been suggested as a possible treatment for peak-dose LID in PD as clinical experience in schizophrenia has shown a lower propensity to induce extra-pyramidal side effects, including tardive dyskinesia and parkinsonism, when used in the treatment of psychosis. This 'atypical' antipsychotic effect has been suggested to be due to changes in the distribution and affinity of dopamine D₂ receptor binding as well as affinity for non-dopaminergic receptors, including 5-HT_{2A/2C}, amongst others (Kapur and Seeman, 2001).

Clozapine (0.1 mg/kg) significantly reduces LID in the MPTP-lesioned primate, without exacerbating parkinsonism. However, higher doses of clozapine (1.0 mg/kg) produce a worsening

parkinsonism (Grondin et al., 1999). In contrast, in another study in MPTP-lesioned primates, the same dose of clozapine, significantly reduced LID without a worsening of PD (Visanji et al., 2006). Quetiapine at relatively high doses (4.0–4.5 mg/kg) significantly reduces LID in MPTP-lesioned primate, without affecting parkinsonism (Oh et al., 2002; Visanji et al., 2006). 8-Methyl-6-(4-methyl-1-piperazinyl)-11H-pyrido[2,3-b][1,4]benzodiazepine (JL-18), an analogue of clozapine has also been shown to reduce dyskinesia without worsening parkinsonism (Hadj Tahar et al., 2000) but this may also relate to dopamine D₄ receptor antagonism properties of this compound. It is clear that a therapeutic window exists whereby clozapine and quetiapine can reduce LID without exacerbating parkinsonism, however, that window may be narrow and variable between species and models.

Clinical trials of 5-HT_{2A} antagonists in LID

A double-blind RCT in PD patients with dyskinesia using low dose clozapine (39.4 ± 4.5 mg/day) reduced on-time with dyskinesia as well as the severity of dyskinesia in response to a single dose of L-DOPA in a 10-week placebo-controlled trial (Durif et al., 2004). However, on clinical evaluation, only dyskinesia at rest, but not activated (as with talking, performing various activities) were reduced and so it is not clear whether this effect will translate into a meaningful improvement in disability caused by dyskinesia in practice. In addition, use of clozapine requires mandatory blood monitoring due to the rare potential risk of agranulocytosis. A prospective double-blind randomised trial of quetiapine failed to demonstrate anti-dyskinetic effect of low dose (25 mg) quetiapine compared to placebo, with a robust placebo effect noted (Katzenschlager et al., 2004). Eight patients continued on the open-label phase at 50 mg daily, but the slight reduction in dyskinesia were usually outweighed by side effects of somnolence and eventually only two patients elected to continue treatment.

ACP-103 (pimavanserin), a 5-HT_{2A} inverse agonist, is currently undergoing evaluation. A double-blind, RCT Phase II trial in 12 PD patients

with LID and motor complications demonstrated good tolerability and reduced dyskinesia, without worsening of parkinsonian symptoms (Roberts, 2006). Further details are yet to be published.

5-HT_{2C} receptors

5-HT_{2C} receptors are selectively localised within the output regions of the basal ganglia, SNr and GPM, with moderate levels in the subthalamic nucleus (STN) and caudate (Hoyer et al., 1986). This suggests a potential role in modulation of basal ganglia function, and possibly movement control. In particular, due to a selective localisation within the CNS, 5-HT_{2C} ligands may have potential therapeutic value without causing systemic side-effects, such as effects on blood pressure. 5-HT_{2C} knockout mice exhibit hyperlocomotion (Tecott et al., 1995) and systemic administration of the putative 5-HT_{2C} agonist, 1-(m-chlorophenyl)piperazine (mCPP) induces hypolocomotion and anxiety in normal rodents (Kennett and Curzon, 1988). Increased 5-HT_{2C} receptor binding has been demonstrated in the SNr in postmortem brain tissue from patients with PD (Fox and Brotchie, 2000a). However, the association with LID is unknown.

Preclinical studies of 5-HT_{2C} antagonists in animal models of LID

To date, preclinical studies in 6-OHDA-lesioned rats of subtype selective 5-HT_{2C} receptor antagonist have only assessed effects on anti-parkinsonian action. Thus, systemic administration of selective 5-HT_{2C} antagonists to 6-OHDA-lesioned rodents potentiates the anti-parkinsonian action of dopamine D₁ and D₂ agonists (Fox and Brotchie, 1996, 2000b) an action mediated via 5-HT_{2C} receptors in the SNr (Fox et al., 1998). Studies in the MPTP-primate model of PD have so far not been possible due to side-effects, including epileptic seizures, with currently available 5-HT_{2C} antagonists (unpublished observations).

The potential mechanism whereby 5-HT_{2C} ligands may modulate LID remains unclear. 5-HT via 5-HT_{2C} receptors are excitatory in the

SNr (Rick et al., 1995; Invernizzi et al., 2007). Thus, according to the current model of basal ganglia function in LID, agonists at 5-HT_{2C} receptors may have an anti-dyskinetic effect. The alternative site of action may be in the striatum, where there is a close link between 5-HT_{2C} receptor activation and dopamine signalling (Di Matteo et al., 2001), but the relationship to striatal function in the generation of LID in PD remains unclear. In addition 5-HT_{2C} receptors are also located with the STN and are excitatory (Stanford et al., 2005). The STN plays a key role in the pathophysiology of PD and LID; indeed the treatment of choice for LID in advanced PD is bilateral implantation of deep brain stimulating (DBS) electrodes into the STN. This results in a marked reduction in LID, due in part to a reduction in dopamine replacement drugs but also due to altered basal ganglia physiology with a 'depriming' effect (Moro et al., 2002). Both systemic administration and local unilateral infusion of mCPP into the STN of normal rodents increased orofacial movements, an effect blocked by the selective 5-HT_{2C} antagonist SDZ SER 082 (Eberle-Wang et al., 1996). However, the role of 5-HT_{2C}-mediated action in the parkinsonian STN is unclear as systemic administration of the 5-HT_{2C} agonist, mCPP to 6-OHDA-lesioned rats enhanced oral movements compared to normal non-parkinsonian animals, but there was no effect with intra-subthalamic administration of mCPP (De Deurwaerdere and Chesselet, 2000). In addition, following the nigrostriatal lesion with 6-OHDA, mCPP-induced Fos expression remained unchanged in the STN but was reduced in the striatum and was markedly enhanced in the entopeduncular nucleus (rodent equivalent of GPM), suggesting that 5-HT_{2C}-mediated oral dyskinesia is not mediated via an action in the STN (De Deurwaerdere and Chesselet, 2000). Indeed, it is more likely that these effects of 5-HT_{2C} within the STN are a model for oral stereotypies than for LID.

To date, no trials in PD and LID of 5-HT_{2C} -selective antagonists have been performed due to lack of clinically available agents. However, the atypical antipsychotics, clozapine and quetiapine are both mixed 5-HT_{2A} and 5-HT_{2C} receptor

antagonists (see above, Table 1) and have potential to reduce LID without worsening PD.

Serotonin transporter

The above discussion highlights the involvement of multiple 5-HT receptors in the genesis of LID. This raises the possibility that it might be valuable to manipulate more than one receptor at once. In the 6-OHDA-lesioned rat, co-treatment with sub-threshold doses of the 5-HT_{1A} agonist, 8-OH-DPAT and 5-HT_{1B} agonist, CP-94253 significantly reduced L-DOPA-induced AIMS, suggested combined actions may be more effective (Carta et al., 2007). The actions of MDMA in MPTP-primates also highlight this approach, it being clear that an MDMA-induced reduction in LID results from a combination of 5-HT_{1A} and 5-HT_{1B} stimulation. One approach to providing stimulation of multiple 5-HT receptors might be to generally enhance synaptic 5-HT levels using an SSRI.

Preclinical studies of SSRIs in animal models of LID

The SSRI, 5-MDOT, can reduce LID in the 6-OHDA-lesioned rats (Henry et al., 1998). Studies in the MPTP-lesioned macaque with 5-MDOT showed a significant reduction in LID but worsening PD motor scores (Gomez-Mancilla and Bedard, 1993). The SSRI, fluvoxamine was ineffective against LID in the MPTP-marmoset (Iravani et al., 2003). The mechanism of action of SSRIs in reducing LID is unclear. However, studies using fluoxetine have shown a reduction in extracellular dopamine following L-DOPA treatment, an effect blocked by a selective 5-HT_{1A} antagonists, suggesting SSRIs reduce efflux of exogenous L-DOPA-derived dopamine (Yamato et al., 2001). This has also been suggested as the potential mechanism whereby SSRIs have been reported in cases to potentially worsen PD symptoms; however, large epidemiological studies have not suggested any increased risk of worsening PD with SSRIs when prescribed for depression in PD (Arbouw et al., 2007).

Clinical studies of SSRIs in LID

The SSRI, fluoxetine, has been shown to reduce apomorphine-induced dyskinesia by 47% in seven PD patients in an open-label study (Durif et al., 1995). Further studies using fluoxetine as a long-term treatment for LID have not been performed. A related SSRI, paroxetine, did not improve L-DOPA-induced dyskinesia (Chung et al., 2005).

Conclusions

Serotonergic agents appear to have potential to reduce LID in PD patients. Multiple subtypes of 5-HT receptors are located within the basal ganglia and are involved in motor function as suggested by preclinical studies. To date, however, clinical studies have been disappointing, as despite showing good reduction in LID, many agents have shown exacerbation of PD motor scores. Some of these problems may have been due to additional pharmacological properties of available agents, e.g. antagonists at dopamine D₂ receptors. This suggests that future development requires subtype selective 5-HT ligands. The use of non-dopaminergic therapies, such as 5-HT, to reduce LID remains a potential option to enable continued use of L-DOPA, as the most effective therapeutic drug for PD.

References

- Antonelli, T., Fuxe, K., Tomasini, M.C., Bartoszyk, G.D., Seyfried, C.A., Tanganelli, S. and Ferraro, L. (2005) Effects of sarizotan on the corticostriatal glutamate pathways. *Synapse*, 58: 193–199.
- Arbouw, M.E., Movig, K.L., Neef, C., Guchelaar, H.J. and Egberts, T.C. (2007) Influence of initial use of serotonergic antidepressants on antiparkinsonian drug use in levodopa-using patients. *Eur. J. Clin. Pharmacol.*, 63: 181–187.
- Bara-Jimenez, W., Bibbiani, F., Morris, M.J., Dimitrova, T., Sherzai, A., Mouradian, M.M. and Chase, T.N. (2005) Effects of serotonin 5-HT_{1A} agonist in advanced Parkinson's disease. *Mov. Disord.*, 20: 932–936.
- Bartoszyk, G.D., Van Amsterdam, C., Greiner, H.E., Rautenberg, W., Russ, H. and Seyfried, C.A. (2004) Sarizotan, a serotonin 5-HT_{1A} receptor agonist and dopamine receptor ligand 1: neurochemical profile. *J. Neural. Transm.*, 111: 113–126.

- Bezard, E., Brotchie, J.M. and Gross, C.E. (2001) Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. *Nat. Rev. Neurosci.*, 2: 577–588.
- Bibbiani, F., Oh, J.D. and Chase, T.N. (2001) Serotonin 5-HT_{1A} agonist improves motor complications in rodent and primate parkinsonian models. *Neurology*, 57: 1829–1834.
- Bishop, C., Kamdar, D.P. and Walker, P.D. (2003) Intrastriatal serotonin 5-HT₂ receptors mediate dopamine D₁-induced hyperlocomotion in 6-hydroxydopamine-lesioned rats. *Synapse*, 50: 164–170.
- Bishop, C., Taylor, J.L., Kuhn, D.M., Eskow, K.L., Park, J.Y. and Walker, P.D. (2006) MDMA and fenfluramine reduce L-DOPA-induced dyskinesia via indirect 5-HT_{1A} receptor stimulation. *Eur. J. Neurosci.*, 23: 2669–2676.
- Bonifati, V., Fabrizio, E., Cipriani, R., Vanacore, N. and Meco, G. (1994) Buspirone in levodopa-induced dyskinesias. *Clin. Neuropharmacol.*, 17: 73–82.
- Brotchie, J.M. (2000) The neural mechanisms underlying levodopa-induced dyskinesia in Parkinson's disease. *Ann. Neurol.*, 47: S105–S112.
- Brotchie, J.M. (2005) Nondopaminergic mechanisms in levodopa-induced dyskinesia. *Mov. Disord.*, 20: 919–931.
- Brunner, D., Buhot, M.C., Hen, R. and Hofer, M. (1999) Anxiety, motor activation, and maternal-infant interactions in 5-HT_{1B} knockout mice. *Behav. Neurosci.*, 113: 587–601.
- Carlsson, T., Carta, M., Winkler, C., Bjorklund, A. and Kirik, D. (2007) Serotonin neuron transplants exacerbate L-DOPA-induced dyskinesias in a rat model of Parkinson's disease. *J. Neurosci.*, 27: 8011–8022.
- Carta, M., Carlsson, T., Kirik, D. and Bjorklund, A. (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain*, 130: 1819–1833.
- Castro, M.E., Pascual, J., Romon, T., Berciano, J., Figols, J. and Pazos, A. (1998) 5-HT_{1B} receptor binding in degenerative movement disorders. *Brain Res.*, 790: 323–328.
- Chase, T.N. (1998) Levodopa therapy: consequences of the nonphysiologic replacement of dopamine. *Neurology*, 50: S17–S25.
- Chen, C.P., Alder, J.T., Bray, L., Kingsbury, A.E., Francis, P.T. and Foster, O.J. (1998) Post-synaptic 5-HT_{1A} and 5-HT_{2A} receptors are increased in Parkinson's disease neocortex. *Ann. N. Y. Acad. Sci.*, 861: 288–289.
- Chung, K.A., Carlson, N.E. and Nutt, J.G. (2005) Short-term paroxetine treatment does not alter the motor response to levodopa in PD. *Neurology*, 64: 1797–1798.
- Crossman, A.R. (1990) A hypothesis on the pathophysiological mechanisms that underlie levodopa- or dopamine agonist-induced dyskinesia in Parkinson's disease: implications for future strategies in treatment. *Mov. Disord.*, 5: 100–108.
- De Deurwaerdere, P. and Chesselet, M.F. (2000) Nigrostriatal lesions alter oral dyskinesia and c-Fos expression induced by the serotonin agonist 1-(m-chlorophenyl)piperazine in adult rats. *J. Neurosci.*, 20: 5170–5178.
- Dekundy, A., Lundblad, M., Danysz, W. and Cenci, M.A. (2007) Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behav. Brain Res.*, 179: 76–89.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22: 229–232.
- Dodel, R.C., Berger, K. and Oertel, W.H. (2001) Health-related quality of life and healthcare utilisation in patients with Parkinson's disease: impact of motor fluctuations and dyskinesias. *Pharmacoeconomics*, 19: 1013–1038.
- Dupre, K.B., Eskow, K.L., Negron, G. and Bishop, C. (2007) The differential effects of 5-HT_{1A} receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat. *Brain Res.*, 1158: 135–143.
- Durif, F., Debilly, B., Galitzky, M., Morand, D., Viallet, F., Borg, M., Thobois, S., Broussolle, E. and Rascol, O. (2004) Clozapine improves dyskinesias in Parkinson disease: a double-blind, placebo-controlled study. *Neurology*, 62: 381–388.
- Durif, F., Vidailhet, M., Bonnet, A.M., Blin, J. and Agid, Y. (1995) Levodopa-induced dyskinesias are improved by fluoxetine. *Neurology*, 45: 1855–1858.
- Eberle-Wang, K., Lucki, I. and Chesselet, M.F. (1996) A role for the subthalamic nucleus in 5-HT_{2C}-induced oral dyskinesia. *Neuroscience*, 72: 117–128.
- Eskow, K.L., Gupta, V., Alam, S., Park, J.Y. and Bishop, C. (2007) The partial 5-HT_{1A} agonist buspirone reduces the expression and development of L-DOPA-induced dyskinesia in rats and improves L-DOPA efficacy. *Pharmacol. Biochem. Behav.*, 87: 306–314.
- Fox, S. and Brotchie, J. (1996) Normethylclozapine potentiates the action of quinpirole in the 6-hydroxydopamine lesioned rat. *Eur. J. Pharmacol.*, 301: 27–30.
- Fox, S.H. and Brotchie, J.M. (2000a) 5-HT_{2C} receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Mov. Disord.*, 15: 1064–1069.
- Fox, S.H. and Brotchie, J.M. (2000b) 5-HT_{2C} receptor antagonists enhance the behavioural response to dopamine D(1) receptor agonists in the 6-hydroxydopamine-lesioned rat. *Eur. J. Pharmacol.*, 398: 59–64.
- Fox, S.H., Lang, A.E. and Brotchie, J.M. (2006) Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: keys to success and roads to failure. *Mov. Disord.*, 21: 1578–1594.
- Fox, S.H., Moser, B. and Brotchie, J.M. (1998) Behavioral effects of 5-HT_{2C} receptor antagonism in the substantia nigra zona reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp. Neurol.*, 151: 35–49.
- Frechilla, D., Cobreros, A., Saldise, L., Moratalla, R., Insausti, R., Luquin, M. and Del Rio, J. (2001) Serotonin 5-HT_{1A} receptor expression is selectively enhanced in the striosomal compartment of chronic parkinsonian monkeys. *Synapse*, 39: 288–296.
- Glennon, J.C., Van Scharrenburg, G., Ronken, E., Hesselink, M.B., Reinders, J.H., Van Der Neut, M., Long, S.K., Feenstra, R.W. and McCreary, A.C. (2006) In vitro

- characterization of SLV308 (7-[4-methyl-1-piperazinyl]-2(3H)-benzoxazolone, monohydrochloride): a novel partial dopamine D2 and D3 receptor agonist and serotonin 5-HT1A receptor agonist. *Synapse*, 60: 599–608.
- Goetz, C.G., Damier, P., Hicking, C., Laska, E., Muller, T., Olanow, C.W., Rascol, O. and Russ, H. (2007) Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. *Mov. Disord.*, 22: 179–186.
- Goetz, C.G., Laska, E., Hicking, C., Damier, P., Muller, T., Nutt, J., Warren Olanow, C., Rascol, O. and Russ, H. (2008) Placebo influences on dyskinesia in Parkinson's disease. *Mov. Disord.*, 23: 700–707.
- Goetz, C.G., Stebbins, G.T., Shale, H.M., Lang, A.E., Chernik, D.A., Chmura, T.A., Ahlskog, J.E. and Dorflinger, E.E. (1994) Utility of an objective dyskinesia rating scale for Parkinson's disease: inter- and intra-rater reliability assessment. *Mov. Disord.*, 9: 390–394.
- Gomez-Mancilla, B. and Bedard, P.J. (1993) Effect of non-dopaminergic drugs on L-DOPA-induced dyskinesias in MPTP-treated monkeys. *Clin. Neuropharmacol.*, 16: 418–427.
- Gordon, P.H., Pullman, S.L., Louis, E.D., Frucht, S.J. and Fahn, S. (2002) Mirtazapine in Parkinsonian tremor. *Parkinsonism Relat. Disord.*, 9: 125–126.
- Gronin, R., Doan, V.D., Gregoire, L. and Bedard, P.J. (1999) D1 receptor blockade improves L-DOPA-induced dyskinesia but worsens parkinsonism in MPTP monkeys. *Neurology*, 52: 771–776.
- Guy, W. (1976) ECDEU Assessment Manual. Rockville, MD: US Department of Health, Education and Welfare. 1976: 534–537.
- Hadj Tahar, A., Belanger, N., Bangassoro, E., Gregoire, L. and Bedard, P.J. (2000) Antidyskinetic effect of JL-18, a clozapine analog, in parkinsonian monkeys. *Eur. J. Pharmacol.*, 399: 183–186.
- Halliday, G.M., Blumbergs, P.C., Cotton, R.G., Blessing, W.W. and Geffen, L.B. (1990) Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. *Brain Res.*, 510: 104–107.
- Henry, B., Crossman, A.R. and Brotchie, J.M. (1998) Characterization of enhanced behavioral responses to L-DOPA following repeated administration in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp. Neurol.*, 151: 334–342.
- Henry, B., Fox, S.H., Crossman, A.R. and Brotchie, J.M. (2001) Mu- and delta-opioid receptor antagonists reduce levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. *Exp. Neurol.*, 171: 139–146.
- Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M. (1986) Serotonin receptors in the human brain II: characterization and autoradiographic localization of 5-HT1C and 5-HT2 recognition sites. *Brain Res.*, 376: 97–107.
- Invernizzi, R.W., Pierucci, M., Calcagno, E., Di Giovanni, G., Di Matteo, V., Benigno, A. and Esposito, E. (2007) Selective activation of 5-HT(2C) receptors stimulates GABA-ergic function in the rat substantia nigra pars reticulata: a combined in vivo electrophysiological and neurochemical study. *Neuroscience*, 144: 1523–1535.
- Iravani, M.M., Jackson, M.J., Kuoppamaki, M., Smith, L.A. and Jenner, P. (2003) 3,4-methylenedioxyamphetamine (ecstasy) inhibits dyskinesia expression and normalizes motor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. *J. Neurosci.*, 23: 9107–9115.
- Iravani, M.M., Tayarani-Binazir, K., Chu, W.B., Jackson, M.J. and Jenner, P. (2006) In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates, the selective 5-hydroxytryptamine 1a agonist (R)-(+)-8-OHDPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. *J. Pharmacol. Exp. Ther.*, 319: 1225–1234.
- Jackson, M.J., Al-Barghouthy, G., Pearce, R.K., Smith, L., Hagan, J.J. and Jenner, P. (2004) Effect of 5-HT1B/D receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. *Pharmacol. Biochem. Behav.*, 79: 391–400.
- Jenner, P. (2003) The MPTP-treated primate as a model of motor complications in PD: primate model of motor complications. *Neurology*, 61: S4–S11.
- Johnston, T.H. and Brotchie, J.M. (2006) Drugs in development for Parkinson's disease: an update. *Curr. Opin. Investig. Drugs*, 7: 25–32.
- Kannari, K., Kurahashi, K., Tomiyama, M., Maeda, T., Arai, A., Baba, M., Suda, T. and Matsunaga, M. (2002) Tandospirone citrate, a selective 5-HT1A agonist, alleviates L-DOPA-induced dyskinesia in patients with Parkinson's disease. *No. To. Shinkei*, 54: 133–137.
- Kannari, K., Yamato, H., Shen, H., Tomiyama, M., Suda, T. and Matsunaga, M. (2001) Activation of 5-HT(1A) but not 5-HT(1B) receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. *J. Neurochem.*, 76: 1346–1353.
- Kapur, S. and Seeman, P. (2001) Does fast dissociation from the dopamine D(2) receptor explain the action of atypical antipsychotics?: a new hypothesis. *Am. J. Psychiatry*, 158: 360–369.
- Katzenschlager, R., Manson, A.J., Evans, A., Watt, H. and Lees, A.J. (2004) Low dose quetiapine for drug induced dyskinesias in Parkinson's disease: a double blind cross over study. *J. Neurol. Neurosurg. Psychiatry*, 75: 295–297.
- Kennett, G.A. and Curzon, G. (1988) Evidence that mCPP may have behavioural effects mediated by central 5-HT1C receptors. *Br. J. Pharmacol.*, 94: 137–147.
- Kish, S.J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L.J., Guttman, M. and Furukawa, Y. (2008) Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. *Brain*, 131: 120–131.
- Kita, H., Chiken, S., Tachibana, Y. and Nambu, A. (2007) Serotonin modulates pallidal neuronal activity in the awake monkey. *J. Neurosci.*, 27: 75–83.
- Knobelman, D.A., Kung, H.F. and Lucki, I. (2000) Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT(1A) and 5-HT(1B) receptors. *J. Pharmacol. Exp. Ther.*, 292: 1111–1117.

- Kumar, N., Van Gerpen, J.A., Bower, J.H. and Ahlskog, J.E. (2005) Levodopa-dyskinesia incidence by age of Parkinson's disease onset. *Mov. Disord.*, 20: 342–344.
- Lang, A.E. and Lozano, A.M. (1998) Parkinson's disease: second of two parts. *N. Engl. J. Med.*, 339: 1130–1143.
- Lavoie, B. and Parent, A. (1990) Immunohistochemical study of the serotonergic innervation of the basal ganglia in the squirrel monkey. *J. Comp. Neurol.*, 299: 1–16.
- Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N. and Cenci, M.A. (2002) Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur. J. Neurosci.*, 15: 120–132.
- Meco, G., Fabrizio, E., Di Rezzo, S., Alessandri, A. and Pratesi, L. (2003) Mirtazapine in L-DOPA-induced dyskinesias. *Clin. Neuropharmacol.*, 26: 179–181.
- Moro, E., Esselink, R.J., Benabid, A.L. and Pollak, P. (2002) Response to levodopa in parkinsonian patients with bilateral subthalamic nucleus stimulation. *Brain*, 125: 2408–2417.
- Mouradian, M.M., Juncos, J.L., Fabbri, G., Schlegel, J., Bartko, J.J. and Chase, T.N. (1988) Motor fluctuations in Parkinson's disease: central pathophysiological mechanisms, part II. *Ann. Neurol.*, 24: 372–378.
- Müller, T., Olanow, C.W., Nutt, J., Hicking, C., Laska, E. and Russ, H. (2006) The Paddy-2 study: The evaluation of sarizotan for treatment-associated dyskinesia in PD patients. *Mov. Disord.*, 21(Suppl 15): p. S591.
- Ng, K.Y., Chase, T.N., Colburn, R.W. and Kopin, I.J. (1970) L-DOPA-induced release of cerebral monoamines. *Science*, 170: 76–77.
- Ng, K.Y., Chase, T.N., Colburn, R.W. and Kopin, I.J. (1971) Dopamine: stimulation-induced release from central neurons. *Science*, 172: 487–489.
- Nicholson, S.L. and Brochie, J.M. (2002) 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease — opportunities for novel therapeutics to reduce the problems of levodopa therapy. *Eur. J. Neurol.*, 9(Suppl 3): 1–6.
- Numan, S., Lundgren, K.H., Wright, D.E., Herman, J.P. and Seroogy, K.B. (1995) Increased expression of 5-HT₂ receptor mRNA in rat striatum following 6-OHDA lesions of the adult nigrostriatal pathway. *Brain Res. Mol. Brain Res.*, 29: 391–396.
- Obeso, J.A., Rodriguez-Oroz, M.C., Rodriguez, M., DeLong, M.R. and Olanow, C.W. (2000) Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. *Ann. Neurol.*, 47: S22–S32.
- Oh, J.D., Bibbiani, F. and Chase, T.N. (2002) Quetiapine attenuates levodopa-induced motor complications in rodent and primate parkinsonian models. *Exp. Neurol.*, 177: 557–564.
- Olanow, C.W., Damier, P., Goetz, C.G., Mueller, T., Nutt, J., Rascol, O., Serbanescu, A., Deckers, F. and Russ, H. (2004) Multicenter, open-label, trial of sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). *Clin. Neuropharmacol.*, 27: 58–62.
- Pact, V. and Giduz, T. (1999) Mirtazapine treats resting tremor, essential tremor, and levodopa-induced dyskinesias. *Neurology*, 53: p. 1154.
- Parkinson Study Group. (2001) Evaluation of dyskinesias in a pilot, randomized, placebo-controlled trial of remacemide in advanced Parkinson disease. *Arch. Neurol.*, 58: 1660–1668.
- Pearce, R.K., Jackson, M., Smith, L., Jenner, P. and Marsden, C.D. (1995) Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (*Callithrix jacchus*). *Mov. Disord.*, 10: 731–740.
- Rascol, O., Damier, P., Goetz, C.G., Hicking, C., Hock, K., Muller, T., Olanow, C.W. and Russ, H. (2006) A large phase III study to evaluate the safety and efficacy of sarizotan in the treatment of L-dopa-induced-dyskinesia associated with PD: The Paddy-1 study. *Mov. Disord.*, 21(Suppl 15): S492–S493.
- Rick, C.E., Stanford, I.M. and Lacey, M.G. (1995) Excitation of rat substantia nigra pars reticulata neurons by 5-hydroxytryptamine in vitro: evidence for a direct action mediated by 5-hydroxytryptamine_{2C} receptors. *Neuroscience*, 69: 903–913.
- Roberts, C. (2006) ACP-103, a 5-HT_{2A} receptor inverse agonist. *Curr. Opin. Investig. Drugs*, 7: 653–660.
- Santiago, M., Matarredona, E.R., Machado, A. and Cano, J. (1998) Influence of serotonergic drugs on in vivo dopamine extracellular output in rat striatum. *J. Neurosci. Res.*, 52: 591–598.
- Stanford, I.M., Kantaria, M.A., Chahal, H.S., Loucif, K.C. and Wilson, C.L. (2005) 5-Hydroxytryptamine induced excitation and inhibition in the subthalamic nucleus: action at 5-HT_{2C}, 5-HT₄ and 5-HT_{1A} receptors. *Neuropharmacology*, 49: 1228–1234.
- Stanford, I.M. and Lacey, M.G. (1996) Differential actions of serotonin, mediated by 5-HT_{1B} and 5-HT_{2C} receptors, on GABA-mediated synaptic input to rat substantia nigra pars reticulata neurons in vitro. *J. Neurosci.*, 16: 7566–7573.
- Stimmell, G.L., Dopheide, J.A. and Stahl, S.M. (1997) Mirtazapine: an antidepressant with noradrenergic and specific serotonergic effects. *Pharmacotherapy*, 17: 10–21.
- Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T. and Matsunaga, M. (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport*, 10: 631–634.
- Taylor, J.L., Bishop, C., Ullrich, T., Rice, K.C. and Walker, P.D. (2006) Serotonin 2A receptor antagonist treatment reduces dopamine D1 receptor-mediated rotational behavior but not L-DOPA-induced abnormal involuntary movements in the unilateral dopamine-depleted rat. *Neuropharmacology*, 50: 761–768.
- Tecott, L.H., Sun, L.M., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F. and Julius, D. (1995) Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature*, 374: 542–546.
- Tomiyama, M., Kimura, T., Maeda, T., Kannari, K., Matsunaga, M. and Baba, M. (2005) A serotonin 5-HT_{1A} receptor agonist prevents behavioral sensitization to L-DOPA in a rodent model of Parkinson's disease. *Neurosci. Res.*, 52: 185–194.

- Van Gerpen, J.A., Kumar, N., Bower, J.H., Weigand, S. and Ahlskog, J.E. (2006) Levodopa-associated dyskinesia risk among Parkinson disease patients in Olmsted County, Minnesota, 1976–1990. *Arch. Neurol.*, 63: 205–209.
- Verhagen Metman, L., Del Dotto, P., van den Munckhof, P., Fang, J., Mouradian, M.M. and Chase, T.N. (1998) Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology*, 50: 1323–1326.
- Visanji, N.P., Gomez-Ramirez, J., Johnston, T.H., Pires, D., Voon, V., Brotchie, J.M. and Fox, S.H. (2006) Pharmacological characterization of psychosis-like behavior in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Mov. Disord.*, 21: 1879–1891.
- Waeber, C. and Palacios, J.M. (1989) Serotonin-1 receptor binding sites in the human basal ganglia are decreased in Huntington's chorea but not in Parkinson's disease: a quantitative in vitro autoradiography study. *Neuroscience*, 32: 337–347.
- Widner, H. and Defer, G.L. (1999) Dyskinesias assessment: from CAPIT to CAPSIT: core assessment program for intracerebral transplantations: core assessment program for surgical interventional therapies. *Mov. Disord.*, 14(Suppl. 1): 60–66.
- Yamato, H., Kannari, K., Shen, H., Suda, T. and Matsunaga, M. (2001) Fluoxetine reduces L-DOPA-derived extracellular DA in the 6-OHDA-lesioned rat striatum. *Neuroreport*, 12: 1123–1126.
- Zhang, X., Andren, P.E. and Svenningsson, P. (2007) Changes on 5-HT₂ receptor mRNAs in striatum and subthalamic nucleus in Parkinson's disease model. *Physiol. Behav.*, 92: 29–33.

CHAPTER 24

Neurobiological basis of serotonin–dopamine antagonists in the treatment of Gilles de la Tourette syndrome

Thomas D.L. Steeves and Susan H. Fox*

*Division of Neurology, University of Toronto and the Morton and Gloria Shulman Movement Disorders Centre,
Toronto Western Hospital, Ontario, Canada*

Abstract: Tourette syndrome (TS) is a heritable neuropsychiatric disorder that presents in childhood with a constellation of motor and non-motor symptoms. The defining feature of the disorder is the presence of brief, stereotyped, motor or vocal behaviours called tics. Although tics are themselves voluntary, they are typically performed secondary to involuntary sensory symptoms or irresistible urges. TS is therefore said to be a disorder of human volition that likely represents a general failure of inhibition. It shares many features with obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and impulse control disorder with which it is also commonly associated. Much of the anatomic substrate for TS probably lies in the circuits that connect multiple areas of cortex with the basal ganglia and thalamus to subserve motivation, inhibition of behaviour, planning of motor acts and detection of threats. To date, pathological studies of TS have been very few and the number of subjects evaluated too small to reliably elucidate the nature and significance of several reported abnormalities. However, evidence derived from both pharmacological trials and selected functional imaging studies suggests that disturbances of the dopaminergic and serotonergic neurotransmitter systems play a key role in the pathogenesis of TS. At the same time, multiple studies have demonstrated reciprocal interactions between the serotonin and dopamine systems of the brain. This information, when placed in the context of the observed functional imaging abnormalities, may generate further insights into the pathophysiology of TS.

Keywords: Tourette syndrome; tics; obsessive compulsive disorder; attention deficit hyperactivity disorder; dopamine; serotonin

Introduction

Tourette syndrome (TS) is an inherited neuropsychiatric disorder that presents in childhood with a constellation of motor and non-motor symptoms.

Its defining feature, however, is the presence of brief, stereotyped, motor or vocal behaviours called tics. TS is distinguished from other movement disorders in that it is primarily a disorder of volition that may represent a more general failure of inhibition in a variety of domains. Prior to enacting tics, up to 90% of adults (Leckman et al., 1993; Kwak et al., 2003a) and 35% of young children (Banaschewski et al., 2003) report that

*Corresponding author. Tel.: (416) 603 6422;
Fax: (416) 603 5004; E-mail: sfox@uhnresearch.ca

they feel unpleasant sensations or urges that ultimately drive much of their unusual behaviour. Although the trigger for the tic is itself involuntary, the action that results from it is, therefore, at least under partial voluntary control. TS is not rare, and prevalence rates of up to 1–2% in schoolchildren and 0.5% in adults have been reported, although the severe cases that come to the attention of neurologists and psychiatrists are likely considerably less common than this (Hornse et al., 2001; Stern et al., 2005). Although the mode of inheritance for TS is unknown, a concordance rate of 86% in monozygotic twins compared to 20% in dizygotic twins points to the primacy of genetics in the development of the disorder (Price et al., 1985; Hyde et al., 1992).

In the majority of cases, TS is associated with behavioural disturbances such as obsessive compulsive disorder (OCD) (Robertson et al., 1988), attention deficit hyperactivity disorder (ADHD) (Comings and Comings, 1987), impulse dyscontrol disorder and intermittent explosive disorder. All have, as their common feature, impaired inhibition of unwanted behaviours. Within families, there is a particularly strong association between tics and obsessive compulsive behaviour (OCB), and this relationship is also reflected in results from candidate gene studies (Pauls et al., 1986a, b). The close association among the symptoms of TS is seen as well in the clinical evaluation of the disorder, where on history it is often impossible to distinguish the compulsive urges that may precede complex tics from the purely compulsive behaviour more typically seen in OCD. Some authors have proposed that tics and obsessions may each form the ends of a spectrum of tic–OCD behaviour that reflects a common, heritable quantitative trait conferring significant genetic risk for one or both of these behaviours. Accordingly, there has been a recent search for ‘endophenotypes’, as revealed by functional imaging or psychiatric features that may more reliably indicate a common underlying pathophysiology.

Anatomic localization of TS

Functional imaging studies during the active generation of tics have delineated a probable anatomic

substrate for TS, consisting of frontal cortical, paralimbic and striatal regions of the brain (Braun et al., 1993; Eidelberg et al., 1997; Peterson et al., 1998; Stern et al., 2000; Jeffries et al., 2002; Bohlhalter et al., 2006). These areas work within interconnected networks to subserve motor planning, behavioural inhibition, motivation, affect and detection of threats — all aspects of brain function that, to one degree or another, are impaired in TS (Harris et al., 1995; Schuerholz et al., 1996; Mahone et al., 2001). Perhaps influenced by the basal ganglia’s central role in the generation of many movement disorders, considerable efforts have been made to elucidate its contribution to TS. However, a substantial body of evidence has also implicated cortical abnormalities in the disorder. The phenomenology of TS itself would indicate that this might be the case, as tics are under partial voluntary control, suppressible and, conversely, suggestible: all of which suggest some degree of conscious and thus cortical contribution to the phenomenology of TS. More direct evidence from anatomic and functional imaging studies supports this view: Volumetric magnetic resonance imaging (MRI) analyses have revealed significantly larger dorsolateral prefrontal regions in children with TS and significantly smaller ones in adults with the disorder (Peterson et al., 2001); Event-related positron emission tomography (PET) during the active generation of tics has shown preferential activation of the dorsolateral rostral prefrontal cortex among a number of other cortical and subcortical regions (Stern et al., 2000). Conversely, functional MRI (fMRI) during the active suppression of tics has demonstrated increased activation of the prefrontal cortex, in addition to relevant areas of thalamus and basal ganglia (Peterson et al., 1998). Transcranial magnetic stimulation studies have shown that cortical inhibition is significantly reduced in the brains of TS patients compared to controls (Ziemann et al., 1997; George et al., 2001), and postmortem analyses of brains from TS patients (described below) have likewise suggested significant cortical abnormalities.

Pathological studies of TS

Unlike many neurologic disorders, where postmortem examinations have provided valuable

insights into the localization and pathogenesis of dysfunction, the existing pathological studies of TS have been very few and the number of subjects included within each study too small to draw firm conclusions from the observed data. Another potentially confounding variable is that most subjects included in such studies have extensive treatment histories with selective serotonin reuptake inhibitors (SSRIs) and dopamine (DA) receptor antagonists. With the exception of quetiapine (Tarazi et al., 2001), most atypical antipsychotics have been shown in rodent studies to increase D2 and D4 receptors in cortex and limbic areas, and D4 receptors in caudate and putamen (Van Tol et al., 1991; Barnes and McPhillips, 1998; Kapur and Seeman, 2001), while typical antipsychotics increase D2 receptors in the basal ganglia (Tarazi et al., 1998, 2001; Tarazi and Baldessarini, 1999). These caveats noted, a range of abnormalities have been described in the few TS patients studied.

Singer and colleagues, in an evaluation restricted to striatum, first reported [^3H]mazindol binding to DA transporters (DATs) increased by 37% in caudate and 50% in putamen in three TS brains compared to normal controls. High-pressure liquid chromatographic assays of DA and its primary metabolites, homovanillic acid and 3,4-dihydroxyphenylacetic acid were normal. Striatal DA D1 and D2 receptor binding, as measured with [^3H]spiperone, was only slightly altered, a finding attributed to prior treatment with neuroleptics. At the time, the authors speculated that the observed increases in DAT binding may have reflected enhanced DA innervation within the striatum (Singer et al., 1991).

Minzer and colleagues subsequently evaluated frontal cortical and striatal postmortem samples from three individuals with TS, compared to three age- and sex-matched controls (Minzer et al., 2004). They performed semi-quantitative immunoblotting for relative densities of DA receptors (D1, D2), DAT, monoamine terminals [vesicular monoamine transporter type 2 (VMAT2)], vesicular docking and release proteins [vesicle-associated membrane protein 2 (VAMP-2), synaptotagmin, synaptosome-associated protein of 25 kDa (SNAP-25), syntaxin, synaptophysin] as well

as α_{2A} -adrenergic receptors that inhibit DA release. Results showed that prefrontal areas rather than striatum had the greatest number of changes, including increased D2, DAT, VAMP-2 and α_{2A} receptor concentrations, with changes being most marked for the D2 receptor protein in prefrontal areas, to greater than 140% of control values. Although small sample sizes and the confounder of prior treatment preclude firm conclusions, the results nonetheless suggest alterations of dopaminergic neurotransmission in frontal subcortical circuits in TS.

Yet another postmortem study has suggested underlying developmental abnormalities of the basal ganglia that would have the potential to give rise to neurotransmitter imbalances within its circuitry. Kalanithi and colleagues, in a study of three TS brains compared to five normal controls, reported a markedly higher total neuron number as well as an increased proportion of GABAergic interneurons in the globus pallidus pars interna (GPi) of TS brains. Conversely, they found lower numbers and densities of neurons as well as a lower proportion of GABAergic interneurons in the globus pallidus pars externa (GPe) and caudate of TS brains. GABAergic interneurons within the striatum are connected by gap junctions to form a web of inhibitory synapses. The authors speculated that the observed imbalance between striatal and GPi inhibitory interneurons would be consistent with a developmental defect in tangential migration in TS patients (Kalanithi et al., 2005).

An interesting point of comparison to the pathological abnormalities reported in TS is the progressive elaboration of dopaminergic innervation seen during the development of the normal human nigrostriatal system. Haycock and colleagues, in a postmortem series of normal brains, have reported that from birth to 9 years of age, striatal levels of DA and other dopaminergic markers increase two–threefold and then gradually decline from a probable peak in late adolescence (Haycock et al., 2003). These findings agree well with the principles of normal brain development, which is in general characterized by an initial over-elaboration of nerve terminals, followed by pruning. Some authors have therefore suggested that TS may result from an over-elaboration of striatal DA innervation in the

pre-adolescent years, with a subsequent failure of regression during maturation. This hypothesis has the advantage of the developmental time course of dopaminergic innervation paralleling the natural history of tics, which typically begin in the first decade of life, peak between 10 and 14 years and then moderate or remit by the time the patient reaches adulthood. While many of the reported pathological abnormalities in TS are concordant with our clinical understanding of the disorder and suggest further avenues for investigation, they will need to be replicated in larger cohorts and, if possible, in drug-naïve subjects.

Anatomic and circuit models of TS

The standard model of basal ganglia function applied to TS

In his original manuscript published in 1885, Georges Gilles de la Tourette identified no anatomic or pathological causes for the condition and referred scientists interested in the origins of the disease to the field of psychology. For many decades thereafter, psychogenic explanations for the disorder dominated thinking within the field, and only with Bockner's observation in 1959 that neuroleptics effectively improved tics (Bockner, 1959), did significant, sustained interest develop in organic models of TS. The various hypotheses for TS that have been developed since this time can be organized according to the anatomic level of the proposed dysfunction, with the most general explanations positing variations in anatomic or circuit properties, followed by progressively reductionistic approaches invoking a variety of neurochemical imbalances to explain disordered brain function. These neurochemical approaches can in turn be referred back to inform the broader anatomic models of the disorder.

One of the earliest and most basic models of TS is based on Albin and colleagues' two-circuit model of 'direct' and 'indirect' pathways within the basal ganglia (Albin et al., 1989). Over the last decade, a number of modifications have been made to the basic model to account for new findings; however, the overall conception of circuits running

through the basal ganglia to release desired behaviours and inhibit potentially unwanted ones remains largely the same. According to the modified version of this model, the major output of the basal ganglia from the GPi and the substantia nigra pars reticulata (SNpr) is a tonic one that continuously inhibits the thalamocortical circuits regulating motor pattern generators in the cerebral cortex. When a particular cortical motor pattern generator initiates a movement, the cortex sends simultaneous signals via glutaminergic projections to the basal ganglia to release intended movements and also to suppress unintended movements. The release of intended movements within the basal ganglia occurs primarily via a GABAergic inhibitory projection called the 'direct' pathway that runs from striatum to the GPi/SNpr. Decreased activity in the GPi/SNpr allows selective release of thalamocortical circuits controlling the relevant motor pattern generators in the cortex. DA D1 receptors predominate within this direct pathway, and stimulation with DA therefore enhances the release of intended movements.

Simultaneously with the first signal to the striatum, the cortex sends secondary signals to inhibit surrounding or competing motor pattern generators. The major pathway for this activity is likely a 'hyperdirect' pathway that runs from (frontal) cortex to stimulate the subthalamic nucleus (STN), which, via relatively divergent glutaminergic projections, then excites the GPe, GPi and SNpr to suppress the thalamus and inhibit thalamocortical circuits (Parent and Hazrati, 1993). The so-called indirect pathway, which runs from striatum to suppress the GPe and withdraw its inhibition from the STN, also participates in this activity (Albin et al., 1989). DA D2 receptors predominate in the indirect pathway and activation of these receptors suppresses the indirect pathway. This is traditionally why D2 blockade has been thought useful for a variety of hyperkinetic movement disorders, including chorea and tics. Blockade of D2 receptors removes dopaminergic suppression of this pathway, thereby allowing it to inhibit background movements that might otherwise interfere with intended movements.

The model of TS derived from this classical model of basal ganglia function proposes an imbalance between the ‘direct’ pathway releasing intended movements and the ‘indirect’ and ‘hyper-direct’ pathways suppressing involuntary movements via the GPe and the STN. The net effect of this imbalance would be reduced activity of the GPi, which would then inappropriately release thalamocortical motor pattern generators (Albin et al., 1989). Although the preceding discussion has focused on the motor function of the basal ganglia, this region also subserves a variety of cognitive and affective functions through parallel and overlapping pathways. The regulation of these pathways is likely organized and governed according to the same principles as for motor function, with excitatory and inhibitory signals, releasing intended processes and suppressing unintended ones. In this context, it is significant that the four major groups of symptoms associated with TS, i.e. tics, OCD, ADHD and impulse dyscontrol, have impaired inhibition of unwanted thoughts and behaviours as their common feature. This essential observation may reflect a common relationship that exists between the disorder’s clinical manifestations and the nature of disruption to the neural circuits that underlie it.

Centre-surround model of basal ganglia function applied to TS

Another more recent modification to the standard model of basal ganglia function postulates a centre-surround organization of pathways within the basal ganglia to provide a more specific framework to understand the motor and behavioural manifestations of TS (Mink, 2006). The circuit properties of the updated standard model of basal ganglia function remain largely unchanged, with the exception of greater emphasis on the focused release of intended movements within the classical, direct pathway from cortex to striatum. This produces a ‘centre’ of decreased activity in the GPi/SNpr, which selectively disinhibits thalamocortical circuits and releases the relevant motor pattern generators in the cortex. Simultaneously, unwanted motor programmes are inhibited primarily by the ‘hyperdirect’ pathway from the

cortex to the STN, which in turn broadly projects to and further stimulates the relevant portions of the GPi/SNpr to provide a ‘surround’ of inhibition on cortical motor activity. Within this framework, Mink has proposed that tics may be generated by the pathological activation of isolated populations of neurons within the striatum, which project to and inappropriately inhibit basal ganglia output, thus releasing the relevant thalamocortical circuits to generate unwanted movements, thoughts and behaviours.

Striosome and matrix model of basal ganglia function applied to TS

Graybiel and colleagues have described functional and neurochemical sub-compartmentalization within the striatum that further refines our understanding of basal ganglia function (Holt et al., 1997) and that may have implications for TS (Canales and Graybiel, 2000). The afferent connections of the striatum can be differentiated on the basis of their expression of neurochemical markers, including opioid receptors, acetylcholinesterase (AChE) and catecholamines. Most notably, staining for AChE delineates patches of lightly stained tissue designated ‘striosomes’ amidst a ‘matrix’ of more heavily stained striatum. This anatomic distinction appears to have some functional significance, as the classical, direct and indirect pathways run within the matrix while other circuits, primarily connecting limbic areas to the substantia nigra pars compacta (SNpc) to provide direct frontal cortical modulation of the nigrostriatal tract, run in the striosomal compartment. The authors have reported that, within this striosomal compartment, increased expression of immediate early genes correlates with the expression of repetitive behaviours that are seen in mice treated with a variety of stimulants. Graybiel and colleagues have proposed that these repetitive behaviours are a model for human stereotypes, which are voluntary, repetitive movements commonly seen in children with developmental disabilities as well as in some normal children and adults. The authors have proposed that stereotypes, and possibly by extension tics, may result from a metabolic imbalance in the activity of the

striosome and matrix compartments (Mink, 2001; Saka and Graybiel, 2003).

Neural oscillation model of basal ganglia function applied to TS

Another emerging concept in neuroscience that has provided a perspective to view movement disorders is the oscillator model of brain function. Over the past decade, increasing interest has developed in the synchronous oscillations of neuronal firing seen in a variety of structures of the brain, including basal ganglia, limbic system and cerebellum. These oscillations are also known to be disrupted in a variety of movement disorders. Intraoperative microelectrode recordings from patients undergoing deep brain stimulation have revealed that abnormal synchronized oscillatory discharges of the GPi and the STN, in phase with cortical activity, are a common feature of both hypokinetic disorders, such as Parkinson's disease, and hyperkinetic disorders such as chorea, ballismus and dystonia. One of the postulated effects of the synchronous discharges is that they may introduce noise into the cortical motor areas, regardless of whether the tonic output is increased or decreased.

Accordingly, Leckman and colleagues have formulated a hypothesis of TS based on oscillatory models of basal ganglia function (Leckman et al., 2006), postulating in a general way that abnormal oscillator activity within corticostriatal circuits produces abnormal discharges in the GPi that allow transient release of the thalamus from its tonic inhibition. This would in turn lead to ectopic activation of selected groups of cortical pyramidal neurons that could generate overt responses in the form of tics or subliminal perceptions in the form of premonitory urges. Deep brain stimulation (DBS) and lesioning of the GPi can produce rapid improvements in tics (Houeto et al., 2005; Zhang et al., 2005), and it is likely that direct evidence supporting or refuting the oscillator hypothesis of TS will therefore emerge with detailed analyses of intraoperative recordings from TS patients. Although single-unit recordings in TS patients have been reported to show phasic discharges in the GPi that correlate to the electromyographic

(EMG) frequency of abnormal movements (Zhang et al., 2005), to our knowledge, no detailed analyses of synchronous oscillatory discharges in TS have yet been published.

Neurochemical models of TS

Role of 5-HT and DA in TS

Some of the most significant insights into the pathogenesis of TS have come from its clinical pharmacology, and it is primarily from these observations that a number of neurochemical hypotheses of TS first emerged. Tics are most reliably suppressed by DA D2 receptor antagonists; while the OCBs commonly seen in the condition are most reliably improved with SSRIs. These findings point to involvement of the dopaminergic and serotonergic systems at some level in the disorder. However, the evidence for a primary serotonergic contribution to OCD, whether in association with TS or independent from it, is somewhat less clear than the evidence for a dopaminergic contribution to tics. Tics often respond well and rapidly to D2 dopaminergic blockade, and this constitutes strong evidence that some form of dopaminergic dysfunction is a proximal cause of the disorder; OCD symptoms, on the other hand, typically respond far more modestly to SSRIs and require higher doses and more prolonged treatment periods than are needed for depression and anxiety.

The DA hypothesis of TS

The accumulated evidence from pharmacological trials, supported both by select postmortem analyses and more recent functional imaging studies, suggests that abnormalities of dopaminergic transmission play a key role in the pathogenesis of TS. Indeed, since Bockner's initial observation in the 1950s that neuroleptics were effective in the treatment of tics (Bockner, 1959), a role for DA has been widely postulated in TS. This view has provided one of the dominant conceptual frameworks for research into the disease for over four decades. The 'DA hypothesis' of TS, as formally

outlined by Singer in 1981, proposed that some form of excessive dopaminergic activity — in the form of increased postsynaptic receptor density or sensitivity, increased release of DA or hyperinnervation by dopaminergic nerve terminals — was responsible for the major symptoms of the disorder (Singer et al., 1981). The focus of much of the research in the field since this time has been to understand the nature of this abnormality through pathological and functional imaging studies. Yet, in spite of the continued accumulated evidence implicating dopaminergic dysfunction in the disease, a number of seemingly contradictory findings has also meant that the specific nature of this abnormality has never been clearly elucidated. Much of the foundation for the DA hypothesis of TS has therefore continued to rest on the original pharmacological evidence.

DA and the treatment of TS

It is well established that typical, first-generation D2 receptor antagonists, such as pimozide and haloperidol, are among the agents most effective at suppressing tics. A number of double-blind placebo-controlled studies have confirmed their clinical efficacy, with reductions of up to 75% in tic severity and frequency reported (Shapiro et al., 1989; Sallee et al., 1997; Tourette Syndrome Study Group, 1999). These agents have also been reported to partially normalize blood flow to hypo-perfused orbital and anterior medial frontal lobes in TS patients (Lampreave et al., 1998). However, these drugs are often poorly tolerated, although, at equivalent doses, pimozide may be better tolerated than haloperidol (Ross and Moldofsky, 1978; Shapiro and Shapiro, 1984). Fluphenazine and trifluoperazine, traditional antipsychotics of the phenothiazine class, also have beneficial effects on tics similar to those of haloperidol (Borison et al., 1982; Goetz et al., 1984), with less sedation and fewer extrapyramidal side effects (EPS) (Silay et al., 2004).

Since the introduction of the so-called atypical, second-generation antipsychotic drugs, there has been increased interest in the use of these agents in the treatment of TS. Drugs of this class include risperidone, olanzapine, ziprasidone, quetiapine

and clozapine. These drugs have nearly a 10-fold-greater affinity for 5-HT₂ receptors than for DA D2 receptors, but their degree of affinity for D2 receptors may still correlate with the treatment effect in TS. Thus clozapine, which has relatively weak DA D2 antagonist properties and more potent anticholinergic and antiserotonergic effects on 5-HT_{2A}, 5-HT_{2C}, 5-HT₆ and 5-HT₇ receptors (Tarsy et al., 2002), has been shown in a small double-blind placebo-controlled trial to be ineffective in the treatment of tics (Caine et al., 1979). In contrast, risperidone, the agent with strongest D2 antagonist action of all atypical antipsychotics and the greatest dose-dependent risk of producing EPS (Tarsy et al., 2002), has been shown superior to placebo for tic reduction in two trials (Bruggeman et al., 2001; Scahill et al., 2003) and equally effective to pimozide (Bruggeman et al., 2001; Gilbert et al., 2004) and clonidine (Gaffney et al., 2002). Two open-label trials of olanzapine, an atypical antipsychotic with modest antagonistic effects on D1, D2 and D4 receptors as well as 5-HT_{2A} and 5-HT_{2C} receptors, have also suggested benefits (Stamenkovic et al., 2000; Budman et al., 2001). Ziprasidone, an agent with chemical structure and pharmacological properties similar to risperidone but with a lower risk of EPS, has been evaluated in a placebo-controlled study of 28 children and found to be superior to placebo in reducing tics (Sallee et al., 2000). Quetiapine, an agent with relatively low affinity for both DA D2 receptors and 5-HT_{2A} receptors has been reported in an open-label study of 12 children to effectively suppress tics by 30–100% (Mukaddes and Abali, 2003). Although, in general, all these agents have less propensity to cause EPS than traditional neuroleptics, emerging concerns over weight gain and related metabolic abnormalities may yet limit their use (Allison and Casey, 2001; Martin et al., 2004; Meyer and Koro, 2004).

Other second-generation antipsychotics include the more selective D2-blocking drugs, such as tiapride and sulpiride, as well as the partial agonist aripiprazole. Tiapride and sulpiride are unavailable in North America but are commonly used for the treatment of tics in Europe. Tiapride has been shown superior to placebo in a study of 27 children (Eggers et al., 1988). A retrospective study of

sulpiride in 63 TS patients of ages 10–68 years reported a positive response in more than 50% of the sample (Robertson et al., 1990). A case report describing the use of the partial D2 agonist aripiprazole in two patients reported excellent benefit with minimal side effects (Kastrup et al., 2005).

Yet another strategy for reducing dopaminergic neurotransmission is tetrabenazine, an inhibitor of VMAT2. Two open-label studies have suggested the benefits of this agent in treating tics. Of a total of 64 TS patients treated in these two studies, two-thirds showed moderate-to-marked reductions in tic severity and frequency, as assessed by the clinician-rated global severity scale. Side effects included parkinsonism, depression, insomnia, akathisia and, in some instances, dystonia (Jankovic and Orman, 1988; Jankovic and Beach, 1997).

Interestingly, levodopa (Black and Mink, 2000) as well as a number of DA agonists have also been shown to suppress tics. The first observation of a beneficial effect on tics from a dopaminergic agent was reported over 20 years ago in three patients whose tics improved after subcutaneous injections of apomorphine (Feinberg and Carroll, 1979). Pergolide, a mixed D1, D2 and D3 agonist, has been shown in two double-blind placebo-controlled studies to significantly improve tics when used at low doses, i.e. one-tenth the dosages used for treating Parkinson's disease (Gilbert et al., 2003). An initial open-label study in children showed significant improvements in 24 of 32 subjects with a drop "greater than 50% from baseline in Yale Global Tic Severity Scale (YGTSS) ratings". Notably, the effect was most marked in children with associated restless legs syndrome (Lipinski et al., 1997). The interpretation of the placebo-controlled studies is somewhat more challenging. One study used a cross-over design and reported significant declines in the YGTSS in 24 children; however, the observed benefits from both the active drug and placebo did not remain consistent across the two arms of the study, possibly reflecting a stronger placebo effect at the start of treatment. The other study used a parallel design for 54 patients randomized in a 2:1 ratio of treatment with pergolide or placebo. In this study, pergolide reduced tics by approximately

25% as measured with the YGTSS; however, the variance of treatment effect in the small placebo group was considerable, and the study was not adequately powered to detect differences in motor or vocal tic subscales. Ropinirole has also been shown in an open-label study to improve tics (Anca et al., 2004). This agent has a higher selectivity for D3 receptors than D2 receptors and minimal affinity for D1 receptors, suggesting possible preferential involvement of D2 and D3 receptors in the disorder.

The specific mechanism whereby these agents exert their effects on tics remains unknown, but all are felt to decrease "the effects of DA release" in the striatum. The effect of DA blockade can be understood in terms of the standard model of basal ganglia circuitry, whereby blocking postsynaptic D2 receptors in the striatum leads to enhanced activity within the indirect pathway where these receptors predominate, thus reducing thalamic release of glutamate into the cortex and thereby decreasing motor output. Based on the standard model, however, agonists acting at postsynaptic D1 and D2 receptors should worsen tics rather than improve them. To explain the efficacy of these agents, most investigators have therefore postulated preferential activation of presynaptic D2 receptors, which would inhibit release of DA at the nerve terminal. This has never been confirmed; however, the lack of benefits observed with the selective autoreceptor agonist talipexol is inconsistent with this interpretation (Goetz et al., 1994).

Functional imaging of dopaminergic neurotransmission in TS

Functional imaging studies with conventional PET and single photon emission tomography (SPECT) ligands have attempted to define potential abnormalities of either pre- or postsynaptic dopaminergic function, as originally proposed in the DA hypothesis of TS. Most of the early studies in this area have generated equivocal or contradictory findings (George et al., 1994; Turjanski et al., 1994; Malison et al., 1995; Wolf et al., 1996; Wong et al., 1997), although a few have shown more clear evidence for abnormalities, mostly

restricted to presynaptic function. Cheon and colleagues reported increased levels of DAT binding in drug-naïve children, reflecting increased numbers or activity of the transporter in this population (Cheon et al., 2004); however, Stamenkovic and colleagues found no differences in transporter binding in adults, the majority of whom were also drug naïve (Stamenkovic et al., 2001). Ernst and colleagues, using [^{18}F]dihydroxyphenylalanine (DOPA) PET, a marker for subcortical DA terminal activity, reported a 20% higher accumulation in the left caudate and a 53% higher accumulation in the right midbrain in TS patients compared to controls (Ernst et al., 1999). The authors interpreted these results as reflecting an up-regulation of DOPA decarboxylase secondary to potential deficits in a number of elements of dopaminergic neurotransmission. Previously, Turjanski and colleagues, also using [^{18}F]DOPA PET, had found no differences in TS patients, most of whom were on active neuroleptic therapy at the time of the study (Turjanski et al., 1994). Albin and colleagues, using [^{11}C]-labeled dihydro-tetrabenazine, a ligand that binds to VMAT in the dopaminergic nerve terminal, demonstrated evidence for excessive dopaminergic innervation restricted to the right ventral striatum in TS patients (Albin et al., 2003).

Previous attempts to evaluate postsynaptic D2 receptor binding with PET and SPECT techniques have shown no consistent differences between TS patients and control subjects (Turjanski et al., 1994; Wolf et al., 1996; Wong et al., 1997; Muller-Vahl et al., 2000). However, these studies have relied on ligands optimized to visualize receptor binding in the striatum and lacking sufficient affinity to reliably image the lower concentrations of DA receptors present in extrastriatal regions of the brain. Thus, in spite of a substantial body of clinical and functional imaging evidence implicating regions outside the striatum in the generation of tics, the neurotransmitter events in these areas have never been adequately assessed.

Over the last several years, however, the development of a new generation of high-affinity dopaminergic ligands now permits high-resolution evaluations of D2 receptor binding outside the striatum (Farde et al., 1997). Most recently,

Gilbert and colleagues have reported results from a small pilot study of six adults with TS with minimal treatment histories compared to six controls, using binding to [^{18}F]fallypride, a high-affinity D2/D3 antagonist that permits visualization of binding in extrastriatal regions (Gilbert et al., 2006). Although the sample size of this study was small, the results nevertheless showed significantly reduced D2/D3 receptor binding in many regions of the brain evaluated, including frontal cortex, thalamus and hippocampus — all regions of interest in TS. The decreased receptor binding in these regions may reflect a primary reduction in regional D2/D3 receptor concentration or, alternatively, a down-regulation secondary to increased DA release. Further studies will be needed to confirm and extend these intriguing findings and determine the mechanism of the observed results.

Yet another approach in the analysis of dopaminergic function in TS has been to evaluate subcortical DA release. Under specific experimental conditions, such as administration of an amphetamine challenge, radioligand binding can allow quantification of neurotransmitter accumulation, as the changes in the binding of the radioligand are inversely proportional to the changes in the levels of endogenous DA with which the ligand must compete for binding sites (Laruelle, 2000; Aalto et al., 2005). Singer and colleagues were the first to use this approach in TS and demonstrated, with an amphetamine challenge in conjunction with binding of [^{11}C]raclopride, that DA release in the putamina of TS patients was increased by 21% compared to controls (Singer et al., 2002). Most recently, Wong and colleagues, again using this method but with higher resolution PET imaging, reported that DA release in the ventral striatum was 50% greater in 14 TS patients compared 10 control subjects, confirming that excessive striatal DA release may indeed be a primary abnormality in the disorder (Wong et al., 2007).

The tonic-phasic DA hypothesis of TS

To explain the observed increases in striatal DA release in TS patients, Singer first proposed that the underlying disturbance in TS could be a deficiency of tonic DA release, possibly secondary

to overactive reuptake by DATs in striatal nerve terminals (Singer et al., 2002). The principles of this deficiency had been first elaborated to explain the observed dopaminergic abnormalities of schizophrenia (Grace, 1991). According to this view, altered neocortical input or overactive DA uptake within the striatum would be modulating abnormally low levels of tonic, extrasynaptic DA release, which in turn would result indirectly in higher levels of phasic, intrasynaptic DA release during spike firing of dopaminergic neurons (Wightman and Robinson, 2002). Potential mechanisms for increased phasic release of DA would include decreased D2 autoreceptor stimulation secondary to the reduced tonic levels of DA outside the synapse. Although Singer's original model focused on striatal abnormalities of DA regulation, considerable evidence has now accrued to implicate widespread serotonergic abnormalities in TS as well. In light of the known interaction between the dopaminergic and serotonergic systems within the brain, updated models have now been proposed that attempt to more effectively integrate current functional imaging findings with the phenomenology and pharmacology of TS (Wong et al., 2007).

Serotonin in TS

The serotonergic system innervates virtually all regions of the CNS, and in particular limbic regions, basal ganglia, anterior cingulate and entorhinal, occipital and frontal cortices. Along with the dopaminergic system, it likely plays important roles in regulating basal ganglia-thalamocortical circuits controlling movement, cognition and affect, and therefore provides a logical substrate to explain the diverse co-morbid psychopathologies of TS. Although the specific role of 5-HT in the pathophysiology of TS remains unclear, serotonergic modulation of dopaminergic neurotransmission would be one mechanism whereby such an interaction could take place.

Evidence for direct serotonergic dysfunction in TS comes from a small number of clinical studies, pharmacological trials as well as more recent efforts at functional imaging. From a purely phenomenological standpoint, one might postulate a role for serotonin in TS, as a number of other

disorders having common evidence for serotonergic dysfunction, including OCD, mood disturbances and migraine, occur at levels several times higher in TS patients than in the general population (Kwak et al., 2003b). A number of clinical studies have also suggested that compared to controls, TS patients have lower blood levels of 5-HT (Leckman et al., 1984; Cath et al., 2001) that treatment with clonidine partially reverses (Leckman et al., 1984). These differences may be more marked in TS patients with co-morbid OCD and also correlate with symptom severity in patients with OCD alone, suggesting that 5-HT may play a more specific role in the OCBs of TS (Cath et al., 2001). TS patients and their family members have also been reported to have lower serotonin/platelet ratios and serum tryptophan levels than controls (Comings, 1990); however, measurements of 5-HT and related metabolites in the cerebrospinal fluid (CSF) of subjects with TS have shown no differences compared to controls (Leckman et al., 1995).

5-HT and the treatment of OCD in TS

No clinical trials have specifically evaluated the treatment of OCD symptoms in TS patients. First-line pharmacological treatment for OCD in the setting of TS is therefore very similar to that for OCD in isolation from it and consists primarily of SSRIs. Although a number of randomized controlled trials (RCTs) have confirmed the efficacy of this approach in OCD, there is little compelling evidence to support the selection of any one of the currently available SSRIs over another as first-line treatment. All but escitalopram have demonstrated efficacy in randomized clinical trials of OCD in adults (Kaplan and Hollander, 2003), and all but citalopram and escitalopram have been evaluated and shown to be effective for OCD in children (March et al., 1998; Geller et al., 2001, 2004; Montgomery et al., 2001; Riddle et al., 2001; Liebowitz et al., 2002). Approximately 40–60% of patients with OCD respond to SSRI therapy, with a mean improvement of 20–40% (Jenike, 2004). The combined use of SSRIs with cognitive behaviour therapy, and in particular exposure and response prevention, has been shown to produce

better, and likely more enduring, therapeutic results (Kampman et al., 2002). Nonetheless, these figures indicate that up to 40% of OCD patients will show no or only a partial treatment response to one or more trials of SSRIs, and this raises the possibility that neurotransmitter systems other than serotonin may be playing more proximal roles in the pathogenesis of the disorder (Stein, 2002). A growing body of molecular imaging and genetic evidence has in fact implicated dopaminergic dysfunction in the pathogenesis of OCD (Insel et al., 1985; Hollander and Wong, 1995; Stein, 2002; Kim et al., 2003; Denys et al., 2004; Pooley et al., 2007), and increasingly, combination treatments (Hollander et al., 2002) and mixed serotonergic/dopaminergic agents, such as risperidone, have been reported helpful in alleviating refractory symptoms of OCD (McDougle et al., 2000; Pfanner et al., 2000).

The mechanism of action of SSRIs in OCD are likely complex, involving a number of the 5-HT receptor subtypes at multiple levels, including postsynaptic cortical 5-HT_{2A}, 5-HT_{2C} and 5-HT₃ receptors and both post- and presynaptic 5-HT_{1A} receptors in cortex and raphe, which regulate the release of serotonin onto projection areas (Kennett et al., 1987; Sprouse and Aghajanian, 1988). One of the primary mechanisms by which SSRIs have traditionally been thought to modulate 5-HT neurotransmission is via desensitization of the somatodendritic serotonin 1A autoreceptors in the midbrain raphe, which ultimately increases serotonin levels in target cortical brain regions (Blier and de Montigny, 1994). Postsynaptic effects of increased serotonin secondary to reduced uptake in target cortical areas may be mediated by desensitization of 5-HT_{2A}, 5-HT_{2C} or 5-HT₃ receptors, as antagonists at these receptors have been reported to reduce both anxiety and OCD symptoms (De Vry, 1995; Millan, 2003; Hackler et al., 2007). The recent finding that disruption of either 5-HT_{2A} or 5-HT_{2C} function in knockout mice reduces anxiety and compulsive-like behaviours also points more specifically to a role for these receptor subtypes in this pathology (Chou-Green et al., 2003; Weisstaub et al., 2006; Heisler et al., 2007). To date, however, no studies have demonstrated changes in 5-HT_{2C}-mediated signalling in TS.

5-HT and the treatment of tics

A specific role for 5-HT in the motor symptoms of TS remains undefined. Two small controlled studies have evaluated the efficacy of the SSRI fluoxetine in treating tics. Both studies found little or no benefit from this agent (Kurlan et al., 1993; Scahill et al., 1997); indeed, anecdotally, these agents may as a class exacerbate tics. As discussed above, a number of the atypical antipsychotics, which have both D2 receptor and 5-HT_{2A/2C} antagonist properties, including risperidone, olanzapine, ziprasidone and quetiapine, have been reported effective in treating tics. The most likely mechanism of action for these agents is still modulation of dopaminergic neurotransmission via D2 receptors, as mixed 5-HT/DA antagonism in dosages sufficient to occupy D2 receptors is usually required to reduce tic severity. However, the ability of these agents to block 5-HT_{2A/2C} receptors suggests a secondary role for serotonin in the pathophysiology of tics. Indeed, a small open-label study using ketanserin, a compound with mainly 5-HT_{2A} antagonist properties and weak D2 antagonist properties (on the order of 200 times less than haloperidol), has also been reported effective in treating tics (Bonnier et al., 1999). Some biological relevance for the use of these agents in TS is also suggested by rodent studies demonstrating that 5-HT_{2A} receptor agonists induce hyperlocomotion (Bishop et al., 2004) while antagonist treatment attenuates it (Higgins et al., 2003). As discussed in other chapters in this book, the serotonergic system modulates dopaminergic neurotransmission in both the 'motor' and 'limbic' basal ganglia-thalamocortical circuits, and this effect may underlie the ability of 5-HT₂ receptor antagonists to reduce symptoms in TS. However, among members of the atypical class, there is no known correlation between 5-HT_{2A} receptor affinity and relative efficacy in the treatment of tics.

Functional imaging of serotonergic neurotransmission in TS

Recent functional imaging analyses applied to evaluate serotonergic neurotransmission in TS have identified clear abnormalities. Behen and

colleagues evaluated tryptophan metabolism in TS patients using [^{11}C]methyl-L-tryptophan (AMT) PET and reported decreased AMT uptake in the dorsolateral prefrontal cortex and increased uptake in caudate and thalamus in TS patients compared to age-matched controls (Behen et al., 2007). Additional differences in regional AMT uptake, correlating with differences in specific behavioural symptoms, were also observed. Those patients with associated OCB had greater uptake in the dorsolateral prefrontal cortex as well as the caudate and lentiform nuclei on the right while those patients with associated ADHD had more uptake in these structures on the left.

SPECT studies of the 5-HT transporter (SERT) using [^{123}I]2-beta-carbomethoxy-3-beta-(4-iodophenyl)-tropane (beta-CIT) in drug-free TS patients have demonstrated decreased binding in the midbrains of subjects with TS compared to controls, reflecting reduced transporter density, affinity or activity in this region that could be secondary to decreased levels of 5-HT. In general, greater reductions in transporter binding have correlated with greater tic severity and OCB (Heinz et al., 1998; Muller-Vahl et al., 2005). Similar studies in patients with OCD but without tics have shown reduced SERT binding in thalamus and midbrain, which again is more pronounced with increasing severity of obsessive compulsive symptomatology, suggesting again that the reduced SERT binding in TS patients may be related specifically to OCD symptoms (Reimold et al., 2007; Zitterl et al., 2007).

Haugbol and colleagues have demonstrated in TS patients significant global elevations in 5-HT_{2A} receptor binding (using [^{18}F]altanserin) in cortical regions and cingulate gyrus as well as subcortically in thalamus, caudate and putamen, and have interpreted these results as reflecting increased density or affinity of available 5-HT_{2A} receptors or a combination of both (Haugbol et al., 2007). The authors reported in this study no specific correlation with OC behaviours, but a prior PET study of OCD patients without tics, using [^{18}F]altanserin, has also demonstrated increased 5-HT_{2A} receptor binding restricted to caudate (Adams et al., 2005). Whether the reported abnormalities of serotonergic function in TS patients are a primary

abnormality or a compensatory one in response to changes in dopaminergic neurotransmission also remains unclear.

Dopamine-serotonin interactions in TS

Multiple studies have demonstrated evidence for reciprocal interactions between serotonin and DA within the brain (Soubrie et al., 1984; Benloucif et al., 1993; Schmidt et al., 1994). PET studies in humans with [^{11}C]raclopride have demonstrated that global activation with the 5-HT_{2A} receptor agonists fenfluramine and psilocybin increases the release of DA in the striatum (Smith et al., 1997; Vollenweider et al., 1999; Kuroki et al., 2003), and more detailed evaluations in rodent models have established that the 5-HT_{2A} and 5-HT_{2C} receptor subtypes exert opposite effects on DA release. In the striatum and the nucleus accumbens, 5-HT_{2A} receptor activation facilitates phasic release of DA in response to stimulus (by regulating either DA synthesis or DA neuron firing rates) but does not influence tonic, basal release of DA (Schmidt et al., 1992; Gudelsky et al., 1994; Schmidt and Fadaye, 1996). Conversely, 5-HT_{2C} receptor activation primarily inhibits stimulus-induced phasic release of DA, although some studies have also suggested that it may inhibit tonic DA release as well (Di Giovanni et al., 1999; Di Matteo et al., 1999; Hutson et al., 2000; Lucas et al., 2000). Given the known relationship between 5-HT_{2A} receptor activation and phasic DA release, Wong and colleagues have proposed that the primary abnormality in TS could be a hypo-serotonergic state producing compensatory up-regulation of postsynaptic 5-HT_{2A} receptors and thus secondary abnormalities of phasic dopaminergic release (Wong et al., 2007).

However, the existing data can equally be interpreted according to reasoning that reverses this thinking. Although low serotonergic states are known to up-regulate dopaminergic function, the converse is also well described. An underlying hypo-dopaminergic state, as originally proposed in the tonic-phasic model of DA regulation, could, in addition to understimulating D2 autoreceptors, equally explain the observed serotonergic abnormalities, which could then also contribute to the

increased phasic release of DA via the activation of up-regulated 5-HT_{2A} receptors. In rodent brains, neonatal depletion of DA in the nigrostriatal system induces up-regulation of 5-HT_{2A} receptors in the striatum (Radja et al., 1993; Laprade et al., 1996; Basura and Walker, 2001) and also enhances 5-HT_{2A} agonist-induced hyperlocomotion and repetitive behaviours, which are in turn reversed with 5-HT_{2A} receptor antagonists (Bishop et al., 2004). Whether the up-regulation of 5-HT_{2A} receptors reported in TS patients is occurring in response to a primary deficiency of tonic DA is not established, but the existence of this form of regulation as revealed by basic physiologic studies suggests that a similar dynamic could be at work in TS.

Conclusions

Evidence derived from both pharmacological trials and selected functional imaging studies suggests that disturbances of the dopaminergic and serotonergic neurotransmitter systems play an essential role in the pathogenesis of TS. Currently, key replicated findings from functional imaging studies have suggested that TS is characterized by increased release of DA in striatum in response to a stimulant challenge, widespread up-regulation of 5-HT_{2A} receptors and decreased binding of the serotonin transporter. The original tonic-phasic model of DA release posited that elevated phasic release of DA in TS was due to understimulation of D2 autoreceptors, which was in turn secondary to low levels of tonic DA release; however, integrating the interactions between the dopaminergic and serotonergic systems into the tonic-phasic model may more adequately account for the current functional imaging findings as well as the phenomenology and pharmacology of TS. Stimulation of up-regulated 5-HT_{2A} receptors may contribute to the elevated phasic release of DA in TS, and the up-regulation of 5-HT_{2A} receptors may in turn be either a primary abnormality or a secondary one, due to the low tonic release of DA, as originally proposed in the tonic-phasic model. These observations suggest new avenues for investigation and treatment of this challenging disorder.

References

- Aalto, S., Bruck, A., Laine, M., Nagren, K. and Rinne, J.O. (2005) Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. *J. Neurosci.*, 25: 2471–2477.
- Adams, K.H., Hansen, E.S., Pinborg, L.H., Hasselbalch, S.G., Svarer, C., Holm, S., Bolwig, T.G. and Knudsen, G.M. (2005) Patients with obsessive-compulsive disorder have increased 5-HT_{2A} receptor binding in the caudate nuclei. *Int. J. Neuropsychopharmacol.*, 8: 391–401.
- Albin, R.L., Koeppe, R.A., Bohnen, N.I., Nichols, T.E., Meyer, P., Wernette, K., Minoshima, S., Kilbourn, M.R. and Frey, K.A. (2003) Increased ventral striatal monoaminergic innervation in Tourette syndrome. *Neurology*, 61: 310–315.
- Albin, R.L., Young, A.B. and Penney, J.B. (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci.*, 12: 366–375.
- Allison, D.B. and Casey, D.E. (2001) Antipsychotic-induced weight gain: a review of the literature. *J. Clin. Psychiatry*, 62(Suppl 7): 22–31.
- Anca, M.H., Giladi, N. and Korczyn, A.D. (2004) Ropinirole in Gilles de la Tourette syndrome. *Neurology*, 62: 1626–1627.
- Banaschewski, T., Woerner, W. and Rothenberger, A. (2003) Premonitory sensory phenomena and suppressibility of tics in Tourette syndrome: developmental aspects in children and adolescents. *Dev. Med. Child Neurol.*, 45: 700–703.
- Barnes, T.R. and McPhillips, M.A. (1998) Novel antipsychotics, extrapyramidal side effects and tardive dyskinesia. *Int. Clin. Psychopharmacol.*, 13(Suppl 3): S49–S57.
- Basura, G.J. and Walker, P.D. (2001) Serotonin 2A receptor regulation of striatal neuropeptide gene expression is selective for tachykinin, but not enkephalin neurons following dopamine depletion. *Brain Res. Mol. Brain Res.*, 92: 66–77.
- Behen, M., Chugani, H.T., Juhasz, C., Helder, E., Ho, A., Maqbool, M., Rothenmel, R.D., Perry, J. and Muzik, O. (2007) Abnormal brain tryptophan metabolism and clinical correlates in Tourette syndrome. *Mov. Disord.*, 22: 2256–2262.
- Benloucif, S., Keegan, M.J. and Galloway, M.P. (1993) Serotonin-facilitated dopamine release in vivo: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 265: 373–377.
- Bishop, C., Tessmer, J.L., Ullrich, T., Rice, K.C. and Walker, P.D. (2004) Serotonin 5-HT_{2A} receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT_{2A/2C} agonism. *J. Pharmacol. Exp. Ther.*, 310: 687–694.
- Black, K.J. and Mink, J.W. (2000) Response to levodopa challenge in Tourette syndrome. *Mov. Disord.*, 15: 1194–1198.

- Blier, P. and de Montigny, C. (1994) Current advances and trends in the treatment of depression. *Trends. Pharmacol. Sci.*, 15: 220–226.
- Bockner, S. (1959) Gilles de la Tourette's disease. *J. Ment. Sci.*, 105: 1078–1081.
- Bohlhalter, S., Goldfine, A., Matteson, S., Garraux, G., Hanakawa, T., Kansaku, K., Wurzman, R. and Hallett, M. (2006) Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. *Brain*, 129: 2029–2037.
- Bonnier, C., Nassogne, M.C. and Evrard, P. (1999) Ketanserin treatment of Tourette's syndrome in children. *Am. J. Psychiatry*, 156: 1122–1123.
- Borison, R.L., Ang, L., Chang, S., Dysken, M., Comaty, J.E. and Davis, J.M. (1982) New pharmacological approaches in the treatment of Tourette syndrome. *Adv. Neurol.*, 35: 377–382.
- Braun, A.R., Stoetter, B., Randolph, C., Hsiao, J.K., Vldar, K., Gernert, J., Carson, R.E., Herscovitch, P. and Chase, T.N. (1993) The functional neuroanatomy of Tourette's syndrome: an FDG-PET study. I. Regional changes in cerebral glucose metabolism differentiating patients and controls. *Neuropsychopharmacology*, 9: 277–291.
- Bruggeman, R., van der Linden, C., Buitelaar, J.K., Gericke, G.S., Hawkrige, S.M. and Temlett, J.A. (2001) Risperidone versus pimozide in Tourette's disorder: a comparative double-blind parallel-group study. *J. Clin. Psychiatry*, 62: 50–56.
- Budman, C.L., Gayer, A., Lesser, M., Shi, Q. and Bruun, R.D. (2001) An open-label study of the treatment efficacy of olanzapine for Tourette's disorder. *J. Clin. Psychiatry*, 62: 290–294.
- Caine, E.D., Polinsky, R.J., Kartzin, R. and Ebert, M.H. (1979) The trial use of clozapine for abnormal involuntary movement disorders. *Am. J. Psychiatry*, 136: 317–320.
- Canales, J.J. and Graybiel, A.M. (2000) A measure of striatal function predicts motor stereotypy. *Nat. Neurosci.*, 3: 377–383.
- Cath, D.C., Spinhoven, P., Landman, A.D. and van Kempen, G.M. (2001) Psychopathology and personality characteristics in relation to blood serotonin in Tourette's syndrome and obsessive-compulsive disorder. *J. Psychopharmacol.*, 15: 111–119.
- Cheon, K.A., Ryu, Y.H., Namkoong, K., Kim, C.H., Kim, J.J. and Lee, J.D. (2004) Dopamine transporter density of the basal ganglia assessed with [123I]IPT SPECT in drug-naïve children with Tourette's disorder. *Psychiatry Res.*, 130: 85–95.
- Chou-Green, J.M., Holscher, T.D., Dallman, M.F. and Akana, S.F. (2003) Repeated stress in young and old 5-HT_{2C} receptor knockout mice. *Physiol. Behav.*, 79: 217–226.
- Comings, D.E. (1990) Blood serotonin and tryptophan in Tourette syndrome. *Am. J. Med. Genet.*, 36: 418–430.
- Comings, D.E. and Comings, B.G. (1987) A controlled study of Tourette syndrome. I. Attention-deficit disorder, learning disorders, and school problems. *Am. J. Hum. Genet.*, 41: 701–741.
- Denys, D., van der Wee, N., Janssen, J., De Geus, F. and Westenberg, H.G. (2004) Low level of dopaminergic D2 receptor binding in obsessive-compulsive disorder. *Biol. Psychiatry*, 55: 1041–1045.
- De Vry, J. (1995) 5-HT_{1A} receptor agonists: recent developments and controversial issues. *Psychopharmacology (Berl.)*, 121: 1–26.
- Di Giovanni, G., De Deurwaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91: 587–597.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (1999) SB 242084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38: 1195–1205.
- Eggers, C., Rothenberger, A. and Berghaus, U. (1988) Clinical and neurobiological findings in children suffering from tic disease following treatment with tiapride. *Eur. Arch. Psychiatry Neurol. Sci.*, 237: 223–229.
- Eidelberg, D., Moeller, J.R., Antonini, A., Kazumata, K., Dhawan, V., Budman, C. and Feigin, A. (1997) The metabolic anatomy of Tourette's syndrome. *Neurology*, 48: 927–934.
- Ernst, M., Zametkin, A.J., Jons, P.H., Matochik, J.A., Pascualvaca, D. and Cohen, R.M. (1999) High presynaptic dopaminergic activity in children with Tourette's disorder. *J. Am. Acad. Child Adolesc. Psychiatry*, 38: 86–94.
- Farde, L., Suhara, T., Nyberg, S., Karlsson, P., Nakashima, Y., Hietala, J. and Halldin, C. (1997) A PET-study of [11C]FLB 457 binding to extrastriatal D2-dopamine receptors in healthy subjects and antipsychotic drug-treated patients. *Psychopharmacology (Berl.)*, 133: 396–404.
- Feinberg, M. and Carroll, B.J. (1979) Effects of dopamine agonists and antagonists in Tourette's disease. *Arch. Gen. Psychiatry*, 36: 979–985.
- Gaffney, G.R., Perry, P.J., Lund, B.C., Bever-Stille, K.A., Arndt, S. and Kuperman, S. (2002) Risperidone versus clonidine in the treatment of children and adolescents with Tourette's syndrome. *J. Am. Acad. Child Adolesc. Psychiatry*, 41: 330–336.
- Geller, D.A., Hoog, S.L., Heiligenstein, J.H., Ricardi, R.K., Tamura, R., Kluszynski, S. and Jacobson, J.G. (2001) Fluoxetine treatment for obsessive-compulsive disorder in children and adolescents: a placebo-controlled clinical trial. *J. Am. Acad. Child Adolesc. Psychiatry*, 40: 773–779.
- Geller, D.A., Wagner, K.D., Emslie, G., Murphy, T., Carpenter, D.J., Wetherhold, E., Perera, P., Machin, A. and Gardiner, C. (2004) Paroxetine treatment in children and adolescents with obsessive-compulsive disorder: a randomized, multicenter, double-blind, placebo-controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry*, 43: 1387–1396.
- George, M.S., Robertson, M.M., Costa, D.C., Ell, P.J., Trimble, M.R., Pilowsky, L. and Verhoeff, N.P. (1994) Dopamine receptor availability in Tourette's syndrome. *Psychiatry Res.*, 55: 193–203.

- George, M.S., Sallee, F.R., Nahas, Z., Oliver, N.C. and Wassermann, E.M. (2001) Transcranial magnetic stimulation (TMS) as a research tool in Tourette syndrome and related disorders. *Adv. Neurol.*, 85: 225–235.
- Gilbert, D.L., Batterson, J.R., Sethuraman, G. and Sallee, F.R. (2004) Tic reduction with risperidone versus pimozide in a randomized, double-blind, crossover trial. *J. Am. Acad. Child Adolesc. Psychiatry*, 43: 206–214.
- Gilbert, D.L., Christian, B.T., Gelfand, M.J., Shi, B., Mantil, J. and Sallee, F.R. (2006) Altered mesolimbocortical and thalamic dopamine in Tourette syndrome. *Neurology*, 67: 1695–1697.
- Gilbert, D.L., Dure, L., Sethuraman, G., Raab, D., Lane, J. and Sallee, F.R. (2003) Tic reduction with pergolide in a randomized controlled trial in children. *Neurology*, 60: 606–611.
- Goetz, C.G., Stebbins, G.T. and Thelen, J.A. (1994) Talipexole and adult Gilles de la Tourette's syndrome: double-blind, placebo-controlled clinical trial. *Mov. Disord.*, 9: 315–317.
- Goetz, C.G., Tanner, C.M. and Klawans, H.L. (1984) Fluphenazine and multifocal tic disorders. *Arch. Neurol.*, 41: 271–272.
- Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, 41: 1–24.
- Gudelsky, G.A., Yamamoto, B.K. and Nash, J.F. (1994) Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT₂ receptor agonists. *Eur. J. Pharmacol.*, 264: 325–330.
- Hackler, E.A., Turner, G.H., Gresch, P.J., Sengupta, S., Deutch, A.Y., Avison, M.J., Gore, J.C. and Sanders-Bush, E. (2007) 5-Hydroxytryptamine_{2C} receptor contribution to m-chlorophenylpiperazine and N-methyl-beta-carboline-3-carboxamide-induced anxiety-like behavior and limbic brain activation. *J. Pharmacol. Exp. Ther.*, 320: 1023–1029.
- Harris, E.L., Schuerholz, L.J., Singer, H.S., Reader, M.J., Brown, J.E., Cox, C., Mohr, J., Chase, G.A. and Denckla, M.B. (1995) Executive function in children with Tourette syndrome and/or attention deficit hyperactivity disorder. *J. Int. Neuropsychol. Soc.*, 1: 511–516.
- Haugbol, S., Pinborg, L.H., Regeur, L., Hansen, E.S., Bolwig, T.G., Nielsen, F.A., Svarer, C., Skovgaard, L.T. and Knudsen, G.M. (2007) Cerebral 5-HT_{2A} receptor binding is increased in patients with Tourette's syndrome. *Int. J. Neuropsychopharmacol.*, 10: 245–252.
- Haycock, J.W., Becker, L., Ang, L., Furukawa, Y., Hornykiewicz, O. and Kish, S.J. (2003) Marked disparity between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. *J. Neurochem.*, 87: 574–585.
- Heinz, A., Knable, M.B., Wolf, S.S., Jones, D.W., Gorey, J.G., Hyde, T.M. and Weinberger, D.R. (1998) Tourette's syndrome: [I-123]beta-CIT SPECT correlates of vocal tic severity. *Neurology*, 51: 1069–1074.
- Heisler, L.K., Zhou, L., Bajwa, P., Hsu, J. and Tecott, L.H. (2007) Serotonin 5-HT_{2C} receptors regulate anxiety-like behavior. *Genes Brain Behav.*, 6: 491–496.
- Higgins, G.A., Enderlin, M., Haman, M. and Fletcher, P.J. (2003) The 5-HT_{2A} receptor antagonist M100,907 attenuates motor and 'impulsive-type' behaviours produced by NMDA receptor antagonism. *Psychopharmacology (Berl.)*, 170: 309–319.
- Hollander, E., Bienstock, C.A., Koran, L.M., Pallanti, S., Marazziti, D., Rasmussen, S.A., Ravizza, L., Benkelfat, C., Saxena, S., Greenberg, B.D., Sasson, Y. and Zohar, J. (2002) Refractory obsessive-compulsive disorder: state-of-the-art treatment. *J. Clin. Psychiatry*, 63(Suppl 6): 20–29.
- Hollander, E. and Wong, C.M. (1995) Obsessive-compulsive spectrum disorders. *J. Clin. Psychiatry*, 56(Suppl 4): 3–6. discussion pp. 53–55.
- Holt, D.J., Graybiel, A.M. and Saper, C.B. (1997) Neurochemical architecture of the human striatum. *J. Comp. Neurol.*, 384: 1–25.
- Hornse, H., Banerjee, S., Zeitlin, H. and Robertson, M. (2001) The prevalence of Tourette syndrome in 13-14-year-olds in mainstream schools. *J. Child Psychol. Psychiatry*, 42: 1035–1039.
- Houeto, J.L., Karachi, C., Mallet, L., Pillon, B., Yelnik, J., Mesnage, V., Welter, M.L., Navarro, S., Pelissolo, A., Damier, P., Pidoux, B., Dormont, D., Cornu, P. and Agid, Y. (2005) Tourette's syndrome and deep brain stimulation. *J. Neurol. Neurosurg. Psychiatry*, 76: 992–995.
- Hutson, P.H., Barton, C.L., Jay, M., Blurton, P., Burkamp, F., Clarkson, R. and Bristow, L.J. (2000) Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT_{2C/2B} receptor antagonists: neurochemical and behavioural studies. *Neuropharmacology*, 39: 2318–2328.
- Hyde, T.M., Aaronson, B.A., Randolph, C., Rickler, K.C. and Weinberger, D.R. (1992) Relationship of birth weight to the phenotypic expression of Gilles de la Tourette's syndrome in monozygotic twins. *Neurology*, 42: 652–658.
- Insel, T.R., Mueller, E.A., Alterman, I., Linnoila, M. and Murphy, D.L. (1985) Obsessive-compulsive disorder and serotonin: is there a connection? *Biol. Psychiatry*, 20: 1174–1188.
- Jankovic, J. and Beach, J. (1997) Long-term effects of tetrabenazine in hyperkinetic movement disorders. *Neurology*, 48: 358–362.
- Jankovic, J. and Orman, J. (1988) Tetrabenazine therapy of dystonia, chorea, tics, and other dyskinesias. *Neurology*, 38: 391–394.
- Jeffries, K.J., Schooler, C., Schoenbach, C., Herscovitch, P., Chase, T.N. and Braun, A.R. (2002) The functional neuroanatomy of Tourette's syndrome: an FDG PET study III: functional coupling of regional cerebral metabolic rates. *Neuropsychopharmacology*, 27: 92–104.
- Jenike, M.A. (2004) Clinical practice. Obsessive-compulsive disorder. *N. Engl. J. Med.*, 350: 259–265.
- Kalanithi, P.S., Zheng, W., Kataoka, Y., DiFiglia, M., Grantz, H., Saper, C.B., Schwartz, M.L., Leckman, J.F. and Vaccarino, F.M. (2005) Altered parvalbumin-positive neuron distribution in basal ganglia of individuals with Tourette syndrome. *Proc. Natl. Acad. Sci. U.S.A.*, 102: 13307–13312.

- Kampman, M., Keijsers, G.P., Hoogduin, C.A. and Verbraak, M.J. (2002) Addition of cognitive-behaviour therapy for obsessive-compulsive disorder patients non-responding to fluoxetine. *Acta Psychiatr. Scand.*, 106: 314–319.
- Kaplan, A. and Hollander, E. (2003) A review of pharmacologic treatments for obsessive-compulsive disorder. *Psychiatr. Serv.*, 54: 1111–1118.
- Kapur, S. and Seeman, P. (2001) Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: a new hypothesis. *Am. J. Psychiatry*, 158: 360–369.
- Kastrup, A., Schlotter, W., Plewnia, C. and Bartels, M. (2005) Treatment of tics in tourette syndrome with aripiprazole. *J. Clin. Psychopharmacol.*, 25: 94–96.
- Kennett, G.A., Marcou, M., Dourish, C.T. and Curzon, G. (1987) Single administration of 5-HT_{1A} agonists decreases 5-HT_{1A} presynaptic, but not postsynaptic receptor-mediated responses: relationship to antidepressant-like action. *Eur. J. Pharmacol.*, 138: 53–60.
- Kim, C.H., Koo, M.S., Cheon, K.A., Ryu, Y.H., Lee, J.D. and Lee, H.S. (2003) Dopamine transporter density of basal ganglia assessed with [123I]IPT SPET in obsessive-compulsive disorder. *Eur. J. Nucl. Med. Mol. Imaging*, 30: 1637–1643.
- Kurlan, R., Como, P.G., Deeley, C., McDermott, M. and McDermott, M.P. (1993) A pilot controlled study of fluoxetine for obsessive-compulsive symptoms in children with Tourette's syndrome. *Clin. Neuropharmacol.*, 16: 167–172.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (2003) 5-HT_{2A} receptor stimulation by DOI, a 5-HT_{2A/2C} receptor agonist, potentiates amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Brain Res.*, 972: 216–221.
- Kwak, C., Dat Vuong, K. and Jankovic, J. (2003a) Premonitory sensory phenomenon in Tourette's syndrome. *Mov. Disord.*, 18: 1530–1533.
- Kwak, C., Vuong, K.D. and Jankovic, J. (2003b) Migraine headache in patients with Tourette syndrome. *Arch. Neurol.*, 60: 1595–1598.
- Lampreave, J.L., Molina, V., Mardomingo, M.J., Bittini, A., Dominguez, P., Almoguera, I., Rubia, F.J. and Carreras, J.L. (1998) Technetium-99m-HMPAO in Tourette's syndrome on neuroleptic therapy and after withdrawal. *J. Nucl. Med.*, 39: 624–628.
- Laprade, N., Radja, F., Reader, T.A. and Soghomonian, J.J. (1996) Dopamine receptor agonists regulate levels of the serotonin 5-HT_{2A} receptor and its mRNA in a subpopulation of rat striatal neurons. *J. Neurosci.*, 16: 3727–3736.
- Laruelle, M. (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J. Cereb. Blood Flow Metab.*, 20: 423–451.
- Leckman, J.F., Anderson, G.M., Cohen, D.J., Ort, S., Harcherik, D.F., Hoder, E.L. and Shaywitz, B.A. (1984) Whole blood serotonin and tryptophan levels in Tourette's disorder: effects of acute and chronic clonidine treatment. *Life Sci.*, 35: 2497–2503.
- Leckman, J.F., Goodman, W.K., Anderson, G.M., Riddle, M.A., Chappell, P.B., McSwiggan-Hardin, M.T., McDougle, C.J., Scahill, L.D., Ort, S.I., Pauls, D.L., et al. (1995) Cerebrospinal fluid biogenic amines in obsessive compulsive disorder, Tourette's syndrome, and healthy controls. *Neuropsychopharmacology*, 12: 73–86.
- Leckman, J.F., Vaccarino, F.M., Kalanithi, P.S. and Rothenberger, A. (2006) Annotation: Tourette syndrome: a relentless drumbeat — driven by misguided brain oscillations. *J. Child Psychol. Psychiatry*, 47: 537–550.
- Leckman, J.F., Walker, D.E. and Cohen, D.J. (1993) Premonitory urges in Tourette's syndrome. *Am. J. Psychiatry*, 150: 98–102.
- Liebowitz, M.R., Turner, S.M., Piacentini, J., Beidel, D.C., Clarvit, S.R., Davies, S.O., Graae, F., Jaffer, M., Lin, S.H., Sallee, F.R., Schmidt, A.B. and Simpson, H.B. (2002) Fluoxetine in children and adolescents with OCD: a placebo-controlled trial. *J. Am. Acad. Child Adolesc. Psychiatry*, 41: 1431–1438.
- Lipinski, J.F., Sallee, F.R., Jackson, C. and Sethuraman, G. (1997) Dopamine agonist treatment of Tourette disorder in children: results of an open-label trial of pergolide. *Mov. Disord.*, 12: 402–407.
- Lucas, G., De Deurwaerdere, P., Porras, G. and Spampinato, U. (2000) Endogenous serotonin enhances the release of dopamine in the striatum only when nigro-striatal dopaminergic transmission is activated. *Neuropharmacology*, 39: 1984–1995.
- Mahone, E.M., Koth, C.W., Cutting, L., Singer, H.S. and Denckla, M.B. (2001) Executive function in fluency and recall measures among children with Tourette syndrome or ADHD. *J. Int. Neuropsychol. Soc.*, 7: 102–111.
- Malison, R.T., McDougle, C.J., van Dyck, C.H., Scahill, L., Baldwin, R.M., Seibyl, J.P., Price, L.H., Leckman, J.F. and Innis, R.B. (1995) [123I]beta-CIT SPECT imaging of striatal dopamine transporter binding in Tourette's disorder. *Am. J. Psychiatry*, 152: 1359–1361.
- March, J.S., Biederman, J., Wolkow, R., Safferman, A., Mardekian, J., Cook, E.H., Cutler, N.R., Dominguez, R., Ferguson, J., Muller, B., Riesenberger, R., Rosenthal, M., Sallee, F.R., Wagner, K.D. and Steiner, H. (1998) Sertraline in children and adolescents with obsessive-compulsive disorder: a multicenter randomized controlled trial. *JAMA*, 280: 1752–1756.
- Martin, A., Scahill, L., Anderson, G.M., Aman, M., Arnold, L.E., McCracken, J., McDougle, C.J., Tierney, E., Chuang, S. and Vitiello, B. (2004) Weight and leptin changes among risperidone-treated youths with autism: 6-month prospective data. *Am. J. Psychiatry*, 161: 1125–1127.
- McDougle, C.J., Epperson, C.N., Pelton, G.H., Wasylink, S. and Price, L.H. (2000) A double-blind, placebo-controlled study of risperidone addition in serotonin reuptake inhibitor-refractory obsessive-compulsive disorder. *Arch. Gen. Psychiatry*, 57: 794–801.
- Meyer, J.M. and Koro, C.E. (2004) The effects of antipsychotic therapy on serum lipids: a comprehensive review. *Schizophr. Res.*, 70: 1–17.

- Millan, M.J. (2003) The neurobiology and control of anxious states. *Prog. Neurobiol.*, 70: 83–244.
- Mink, J.W. (2001) Basal ganglia dysfunction in Tourette's syndrome: a new hypothesis. *Pediatr. Neurol.*, 25: 190–198.
- Mink, J.W. (2006) Neurobiology of basal ganglia and Tourette syndrome: basal ganglia circuits and thalamocortical outputs. *Adv. Neurol.*, 99: 89–98.
- Minzer, K., Lee, O., Hong, J.J. and Singer, H.S. (2004) Increased prefrontal D2 protein in Tourette syndrome: a postmortem analysis of frontal cortex and striatum. *J. Neurol. Sci.*, 219: 55–61.
- Montgomery, S.A., Kasper, S., Stein, D.J., Bang Hedegaard, K. and Lemming, O.M. (2001) Citalopram 20 mg, 40 mg and 60 mg are all effective and well tolerated compared with placebo in obsessive-compulsive disorder. *Int. Clin. Psychopharmacol.*, 16: 75–86.
- Mukaddes, N.M. and Abali, O. (2003) Quetiapine treatment of children and adolescents with Tourette's disorder. *J. Child Adolesc. Psychopharmacol.*, 13: 295–299.
- Muller-Vahl, K.R., Berding, G., Kolbe, H., Meyer, G.J., Hundeshagen, H., Dengler, R., Knapp, W.H. and Emrich, H.M. (2000) Dopamine D2 receptor imaging in Gilles de la Tourette syndrome. *Acta Neurol. Scand.*, 101: 165–171.
- Muller-Vahl, K.R., Meyer, G.J., Knapp, W.H., Emrich, H.M., Gielow, P., Brucke, T. and Berding, G. (2005) Serotonin transporter binding in Tourette syndrome. *Neurosci. Lett.*, 385: 120–125.
- Parent, A. and Hazrati, L.N. (1993) Anatomical aspects of information processing in primate basal ganglia. *Trends Neurosci.*, 16: 111–116.
- Pauls, D.L., Leckman, J.F., Towbin, K.E., Zahner, G.E. and Cohen, D.J. (1986a) A possible genetic relationship exists between Tourette's syndrome and obsessive-compulsive disorder. *Psychopharmacol. Bull.*, 22: 730–733.
- Pauls, D.L., Towbin, K.E., Leckman, J.F., Zahner, G.E. and Cohen, D.J. (1986b) Gilles de la Tourette's syndrome and obsessive-compulsive disorder. Evidence supporting a genetic relationship. *Arch. Gen. Psychiatry*, 43: 1180–1182.
- Peterson, B.S., Skudlarski, P., Anderson, A.W., Zhang, H., Gatenby, J.C., Lacadie, C.M., Leckman, J.F. and Gore, J.C. (1998) A functional magnetic resonance imaging study of tic suppression in Tourette syndrome. *Arch. Gen. Psychiatry*, 55: 326–333.
- Peterson, B.S., Staib, L., Scahill, L., Zhang, H., Anderson, C., Leckman, J.F., Cohen, D.J., Gore, J.C., Albert, J. and Webster, R. (2001) Regional brain and ventricular volumes in Tourette syndrome. *Arch. Gen. Psychiatry*, 58: 427–440.
- Pfanner, C., Marazziti, D., Dell'Osso, L., Presta, S., Gemignani, A., Milanfranchi, A. and Cassano, G.B. (2000) Risperidone augmentation in refractory obsessive-compulsive disorder: an open-label study. *Int. Clin. Psychopharmacol.*, 15: 297–301.
- Pooley, E.C., Fineberg, N. and Harrison, P.J. (2007) The met(158) allele of catechol-O-methyltransferase (COMT) is associated with obsessive-compulsive disorder in men: case-control study and meta-analysis. *Mol. Psychiatry*, 12: 556–561.
- Price, R.A., Kidd, K.K., Cohen, D.J., Pauls, D.L. and Leckman, J.F. (1985) A twin study of Tourette syndrome. *Arch. Gen. Psychiatry*, 42: 815–820.
- Radja, F., Descarries, L., Dewar, K.M. and Reader, T.A. (1993) Serotonin 5-HT₁ and 5-HT₂ receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. *Brain Res.*, 606: 273–285.
- Reimold, M., Smolka, M.N., Zimmer, A., Batra, A., Knobel, A., Solbach, C., Mundt, A., Smolczyk, H.U., Goldman, D., Mann, K., Reischl, G., Machulla, H.J., Bares, R. and Heinz, A. (2007) Reduced availability of serotonin transporters in obsessive-compulsive disorder correlates with symptom severity—a [(11)C]DASB PET study. *J. Neural. Transm.*, 114: 1603–1609.
- Riddle, M.A., Reeve, E.A., Yaryura-Tobias, J.A., Yang, H.M., Claghorn, J.L., Gaffney, G., Greist, J.H., Holland, D., McConville, B.J., Pigott, T. and Walkup, J.T. (2001) Fluvoxamine for children and adolescents with obsessive-compulsive disorder: a randomized, controlled, multicenter trial. *J. Am. Acad. Child Adolesc. Psychiatry*, 40: 222–229.
- Robertson, M.M., Schnieden, V. and Lees, A.J. (1990) Management of Gilles de la Tourette syndrome using sulpiride. *Clin. Neuropharmacol.*, 13: 229–235.
- Robertson, M.M., Trimble, M.R. and Lees, A.J. (1988) The psychopathology of the Gilles de la Tourette syndrome. A phenomenological analysis. *Br. J. Psychiatry*, 152: 383–390.
- Ross, M.S. and Moldofsky, H. (1978) A comparison of pimozide and haloperidol in the treatment of Gilles de la Tourette's syndrome. *Am. J. Psychiatry*, 135: 585–587.
- Saka, E. and Graybiel, A.M. (2003) Pathophysiology of Tourette's syndrome: striatal pathways revisited. *Brain Dev.*, 25(Suppl 1): S15–S19.
- Sallee, F.R., Kurlan, R., Goetz, C.G., Singer, H., Scahill, L., Law, G., Dittman, V.M. and Chappell, P.B. (2000) Ziprasidone treatment of children and adolescents with Tourette's syndrome: a pilot study. *J. Am. Acad. Child Adolesc. Psychiatry*, 39: 292–299.
- Sallee, F.R., Nesbitt, L., Jackson, C., Sine, L. and Sethuraman, G. (1997) Relative efficacy of haloperidol and pimozide in children and adolescents with Tourette's disorder. *Am. J. Psychiatry*, 154: 1057–1062.
- Scahill, L., Leckman, J.F., Schultz, R.T., Katsovich, L. and Peterson, B.S. (2003) A placebo-controlled trial of risperidone in Tourette syndrome. *Neurology*, 60: 1130–1135.
- Scahill, L., Riddle, M.A., King, R.A., Hardin, M.T., Rasmussen, A., Makuch, R.W. and Leckman, J.F. (1997) Fluoxetine has no marked effect on tic symptoms in patients with Tourette's syndrome: a double-blind placebo-controlled study. *J. Child Adolesc. Psychopharmacol.*, 7: 75–85.
- Schmidt, C.J. and Fadaye, G.M. (1996) Regional effects of MK-801 on dopamine release: effects of competitive NMDA or 5-HT_{2A} receptor blockade. *J. Pharmacol. Exp. Ther.*, 277: 1541–1549.

- Schmidt, C.J., Fadayel, G.M., Sullivan, C.K. and Taylor, V.L. (1992) 5-HT₂ receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur. J. Pharmacol.*, 223: 65–74.
- Schmidt, C.J., Sullivan, C.K. and Fadayel, G.M. (1994) Blockade of striatal 5-hydroxytryptamine₂ receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.*, 62: 1382–1389.
- Schuerholz, L.J., Baumgardner, T.L., Singer, H.S., Reiss, A.L. and Denckla, M.B. (1996) Neuropsychological status of children with Tourette's syndrome with and without attention deficit hyperactivity disorder. *Neurology*, 46: 958–965.
- Shapiro, A.K. and Shapiro, E. (1984) Controlled study of pimozide vs. placebo in Tourette's syndrome. *J. Am. Acad. Child Psychiatry*, 23: 161–173.
- Shapiro, E., Shapiro, A.K., Fulop, G., Hubbard, M., Mandeli, J., Nordlie, J. and Phillips, R.A. (1989) Controlled study of haloperidol, pimozide and placebo for the treatment of Gilles de la Tourette's syndrome. *Arch. Gen. Psychiatry*, 46: 722–730.
- Silay, Y., Vuogn, K. and Jankovic, J. (2004) The efficacy and safety of fluphenazine in patients with tourette syndrome. *Neurology*, 62: p. A506.
- Singer, H.S., Hahn, I.H. and Moran, T.H. (1991) Abnormal dopamine uptake sites in postmortem striatum from patients with Tourette's syndrome. *Ann. Neurol.*, 30: 558–562.
- Singer, H.S., Rabins, P., Tune, L.E. and Coyle, J.T. (1981) Serum haloperidol levels in Gilles de la Tourette syndrome. *Biol. Psychiatry*, 16: 79–84.
- Singer, H.S., Szymanski, S., Giuliano, J., Yokoi, F., Dogan, A.S., Brasic, J.R., Zhou, Y., Grace, A.A. and Wong, D.F. (2002) Elevated intrasynaptic dopamine release in Tourette's syndrome measured by PET. *Am. J. Psychiatry*, 159: 1329–1336.
- Smith, G.S., Dewey, S.L., Brodie, J.D., Logan, J., Vitkun, S.A., Simkowitz, P., Schloesser, R., Alexoff, D.A., Hurley, A., Cooper, T. and Volkow, N.D. (1997) Serotonergic modulation of dopamine measured with [¹¹C]raclopride and PET in normal human subjects. *Am. J. Psychiatry*, 154: 490–496.
- Soubrie, P., Reisine, T.D. and Glowinski, J. (1984) Functional aspects of serotonin transmission in the basal ganglia: a review and an in vivo approach using the push-pull cannula technique. *Neuroscience*, 13: 605–625.
- Sprouse, J.S. and Aghajanian, G.K. (1988) Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology*, 27: 707–715.
- Stamenkovic, M., Schindler, S.D., Aschauer, H.N., De Zwaan, M., Willinger, U., Resinger, E. and Kasper, S. (2000) Effective open-label treatment of tourette's disorder with olanzapine. *Int. Clin. Psychopharmacol.*, 15: 23–28.
- Stamenkovic, M., Schindler, S.D., Asenbaum, S., Neumeister, A., Willeit, M., Willinger, U., de Zwaan, M., Riederer, F., Aschauer, H.N. and Kasper, S. (2001) No change in striatal dopamine re-uptake site density in psychotropic drug naive and in currently treated Tourette's disorder patients: a [(123)I]-beta-CIT SPECT-study. *Eur. Neuropsychopharmacol.*, 11: 69–74.
- Stein, D.J. (2002) Obsessive-compulsive disorder. *Lancet*, 360: 397–405.
- Stern, E., Silbersweig, D.A., Chee, K.Y., Holmes, A., Robertson, M.M., Trimble, M., Frith, C.D., Frackowiak, R.S. and Dolan, R.J. (2000) A functional neuroanatomy of tics in Tourette syndrome. *Arch. Gen. Psychiatry*, 57: 741–748.
- Stern, J.S., Burza, S. and Robertson, M.M. (2005) Gilles de la Tourette's syndrome and its impact in the UK. *Postgrad. Med. J.*, 81: 12–19.
- Tarazi, F.I. and Baldessarini, R.J. (1999) Dopamine D4 receptors: significance for molecular psychiatry at the millennium. *Mol. Psychiatry*, 4: 529–538.
- Tarazi, F.I., Yeghiayan, S.K., Neumeyer, J.L. and Baldessarini, R.J. (1998) Medial prefrontal cortical D2 and striatolimbic D4 dopamine receptors: common targets for typical and atypical antipsychotic drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 22: 693–707.
- Tarazi, F.I., Zhang, K. and Baldessarini, R.J. (2001) Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J. Pharmacol. Exp. Ther.*, 297: 711–717.
- Tarsy, D., Baldessarini, R.J. and Tarazi, F.I. (2002) Effects of newer antipsychotics on extrapyramidal function. *CNS Drugs*, 16: 23–45.
- Tourette Syndrome Study Group. (1999) Short-term versus longer term pimozide therapy in Tourette's syndrome: a preliminary study. *Neurology*, 52: 874–877.
- Turjanski, N., Sawle, G.V., Playford, E.D., Weeks, R., Lammerstma, A.A., Lees, A.J. and Brooks, D.J. (1994) PET studies of the presynaptic and postsynaptic dopaminergic system in Tourette's syndrome. *J. Neurol. Neurosurg. Psychiatry*, 57: 688–692.
- Van Tol, H.H., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B. and Civelli, O. (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature*, 350: 610–614.
- Vollenweider, F.X., Vontobel, P., Hell, D. and Leenders, K.L. (1999) 5-HT modulation of dopamine release in basal ganglia in psilocybin-induced psychosis in man — a PET study with [¹¹C]raclopride. *Neuropsychopharmacology*, 20: 424–433.
- Weisstaub, N.V., Zhou, M., Lira, A., Lambe, E., Gonzalez-Maeso, J., Hornung, J.P., Sibille, E., Underwood, M., Itoharu, S., Dauer, W.T., Ansorge, M.S., Morelli, E., Mann, J.J., Toth, M., Aghajanian, G., Sealfon, S.C., Hen, R. and Gingrich, J.A. (2006) Cortical 5-HT_{2A} receptor signaling modulates anxiety-like behaviors in mice. *Science*, 313: 536–540.
- Wightman, R.M. and Robinson, D.L. (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. *J. Neurochem.*, 82: 721–735.
- Wolf, S.S., Jones, D.W., Knable, M.B., Gorey, J.G., Lee, K.S., Hyde, T.M., Coppola, R. and Weinberger, D.R. (1996)

- Tourette syndrome: prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. *Science*, 273: 1225–1227.
- Wong, D.F., Brasic, J.R., Singer, H.S., Schretlen, D.J., Kuwabara, H., Zhou, Y., Nandi, A., Maris, M.A., Alexander, M., Ye, W., Rousset, O., Kumar, A., Szabo, Z., Gjedde, A. and Grace, A.A. (2007) Mechanisms of dopaminergic and serotonergic neurotransmission in Tourette syndrome: clues from an in vivo neurochemistry study with PET. *Neuropsychopharmacology*, 7: p. 7.
- Wong, D.F., Singer, H.S., Brandt, J., Shaya, E., Chen, C., Brown, J., Kimball, A.W., Gjedde, A., Dannals, R.F., Ravert, H.T., Wilson, P.D. and Wagner, H.N., Jr. (1997) D2-like dopamine receptor density in Tourette syndrome measured by PET. *J. Nucl. Med.*, 38: 1243–1247.
- Zhang, X.H., Li, Y.J. and Zhuang, P. (2005) [A study on outcome and mechanism of surgical treatment for Tourette's syndrome]. *Zhonghua Wai Ke Za Zhi*, 43: 608–611.
- Ziemann, U., Paulus, W. and Rothenberger, A. (1997) Decreased motor inhibition in Tourette's disorder: evidence from transcranial magnetic stimulation. *Am. J. Psychiatry*, 154: 1277–1284.
- Zitterl, W., Aigner, M., Stompe, T., Zitterl-Eglseer, K., Gutierrez-Lobos, K., Schmidl-Mohl, B., Wenzel, T., Demal, U., Zetting, G., Hornik, K. and Thau, K. (2007) [123I]-beta-CIT SPECT imaging shows reduced thalamus-hypothalamus serotonin transporter availability in 24 drug-free obsessive-compulsive checkers. *Neuropsychopharmacology*, 32: 1661–1668.

CHAPTER 25

Serotonergic and dopaminergic modulation of attentional processes

Vasileios Boulougouris^{1,*} and Eleftheria Tsaltas²

¹*Department of Experimental Psychology and the Behavioural and Clinical Neuroscience Institute (BCNI),
University of Cambridge, CB2 3EB, Cambridge, UK*

²*Experimental Psychology Laboratory, Department of Psychiatry, Athens University Medical School, Eginition Hospital,
11528 Athens, Greece*

Abstract: Disturbances in attentional processes are a common feature of several psychiatric disorders such as schizophrenia, attention deficit/hyperactivity disorder and Huntington's disease. The use of animal models has been useful in defining various candidate neural systems thus enabling us to translate basic laboratory science to the clinic and vice-versa. In this chapter, a comparative and integrated account is provided on the neuroanatomical and neurochemical modulation of basic behavioural operations such as selective attention, vigilance, set-shifting and executive control focusing on the comparative functions of the serotonin and dopamine systems in the cognitive control exerted by the prefrontal cortex. Specifically, we have reviewed evidence emerging from several behavioural paradigms in experimental animals and humans each of which centres on a different aspect of the attentional function. These paradigms offering both human and animal variants include the five-choice serial reaction time task (5CSRTT), attentional set-shifting and stop-signal reaction time task. In each case, the types of operation that are measured by the given paradigm and their neural correlates are defined. Then, the role of the ascending dopaminergic and serotonergic systems in the neurochemical modulation of its behavioural output are examined, and reference is made to clinical implications for neurological and neuropsychiatric disorders which exhibit deficits in these cognitive tests.

Keywords: attention; 5CSRTT; shifting; inhibition; dopamine; serotonin

Introduction

Attention refers to the processes determining an organism's receptivity to external or internal excitation and hence the probability that it will engage in the processing of that excitation (Parasuraman, 1998). Although it is often treated

as a cognitive function, it is distinct in encompassing a multitude of manifestations which underlie and sustain the activity of the other cognitive functions. Attentional processes facilitate cognitive and behavioural performance in several ways, through the selection and integration of sensory inputs which is essential for efficient learning and remembering, as well as for the organisation of appropriate responses. Impaired attentional processing may therefore become manifested as inattention, distractibility, memory impairment,

*Corresponding author. Tel.: +(0044) 01223 765290;
Fax: +(0044) 01223 333564; E-mail: vb257@cam.ac.uk

confusion, perseveration or disinhibition. Recognition of the diversity of attention has led to the identification of three distinct fundamental qualities: selection, which enables the allocation of priority to certain informational elements to the exclusion of others; vigilance, which refers to the capacity for attentional persistence over time; and control, which optimises performance, for example, by inhibition of concurrent activities (Parasuraman, 1998; Robbins, 2002, 2005).

Impaired attentional processing leads to unfocused cognitive function and consequent failure to regulate behaviour efficiently in response to environmental changes. Behavioural inflexibility may take the form of impulsivity (hasty responding with no regard for consequences) or compulsivity (needless response repetition). Such cognitive and behavioural deregulations are often noted within the normal state, for example in periods of severe stress or fatigue. They are also encountered as a core deficit in several neuropsychiatric abnormalities such as Parkinson's and Huntington's diseases, schizophrenia, attention deficit hyperactivity disorder (ADHD), Alzheimer's disease and drug addiction. In fact, important information on the neuroanatomical and neurochemical substrate of attention has been gained by the study of attentional deficits in these neuropsychiatric conditions, which share attentional and executive deficits reminiscent of those induced by prefrontal lesions.

Given that attentional failures can both compromise normal behaviour and underlie psychopathology, the understanding of the neuroanatomical and neurochemical substrate of the attentional function is crucial both in a theoretical and a clinical context.

Neuropsychiatric disorders involving deficits in attentional processes

The subcortical dementias

Huntington's and Parkinson's diseases are both characterised by attentional deficits. Early deficits in Huntington's disease include impairments in concentration and mental tracking, as well as in set maintenance and shifting (Folstein et al., 1990). Similarly, in Parkinson's disease attentional deficits

initially emerge in complex tasks requiring shifting or sustained attention and mental tracking (Huber and Shuttleworth, 1990; Owen et al., 1992). In both disorders, attention span is spared in the early stages (Brown and Marsen, 1988; Huber and Shuttleworth, 1990). Executive deficits accompanying Huntington's disease also bear similarities to those of prefrontal symptomatology, including impaired behavioural regulation and planning (Folstein et al., 1990). Correspondingly, prefrontal-like executive dysfunctions such as difficulty in response initiation, set maintenance and switching, serial and temporal ordering and executive planning have been reported in Parkinson's patients (Freedman, 1990; Dubois et al., 1991).

Huntington's disease is associated with atrophy of the striatal structures of the caudate nucleus and putamen, while thalamic nuclei and the cerebellum may also be affected. Parkinson's disease involves loss of dopaminergic neurons in the pars compacta of the substantia nigra, accompanied by reduction of dopamine (DA) in the basal ganglia (caudate and putamen). Although Parkinson's disease is mostly associated with DA, other neurotransmitter systems are also involved. Cell loss is noted in the locus coeruleus (noradrenergic source to cortex), the nucleus basalis (major cholinergic input to cortex), the dorsal raphe nucleus, hypothalamus, mamillary bodies and reticular formation.

As mentioned above, the attentional and executive deficits encountered in these subcortical dementias bear similarities to those produced by frontal lobe damage with involvement of the prefrontal cortex (PFC). However, magnetic resonance imaging (MRI) data from Huntington's patients have not revealed specific frontal volume loss (Aylward et al., 1998). It seems likely, therefore, that the prefrontal symptomatology results from disconnection of fronto-striatal loops due to caudate atrophy. Similarly in Parkinson's disease cortical involvement appears to be in part caused by frontal disconnections due to DA loss (Jacobs et al., 2003).

Schizophrenia

Clinical observation of schizophrenic patients outlines a number of attentional disturbances such

as deficits in information selection and utilisation as well as in sustaining and shifting attention in response to changing environmental demands. Compulsive activity or fixation to trivial environmental stimuli may also be evident. Although these marked deficits lead to inappropriate responding and illogical discontinuities in behavioural course, nevertheless impaired attentional processing has only recently been accepted as one of the core deficits of the disorder.

Brain malfunction in schizophrenia is still not understood, though subtle brain abnormalities have been described in schizophrenic patient populations. The hippocampus, entorhinal and cingulate cortices have been implicated by structural and functional neuroimaging data (Tamminga et al., 2002; Pincus and Tucker, 2003) and there are reports of decreased cortical grey matter (Sullivan et al., 1998). Converging evidence suggests frontal lobe dysfunction (Weinberger et al., 1991). It has been proposed that schizophrenia may be the result of dysfunction in the neural circuitry linking the PFC with the thalamus, cerebellum and possibly the basal ganglia (Andreasen et al., 1998).

Attention deficit hyperactivity disorder (ADHD)

This disorder of executive attention is characterised by a persistent pattern of inattention and/or hyperactivity, as well as forgetfulness, poor impulse control and distractibility. It is considered neurodevelopmental due to the apparent lag in the development in impulse control. Frontal as well as striatal abnormalities have been associated with ADHD (Ernst et al., 1998; Rubia et al., 2000; Mehta et al., 2001; Solanto et al., 2001). As in schizophrenia, hypoactivity of the mesocortical DA projection has been implicated in its pathogenesis.

Role of the PFC in the anatomical substrate of attention: fronto-striatal loops

Adaptive behaviour requires selection of responses appropriate to current environmental demands, in tandem with the capacity to suppress responding

which is no longer relevant. The maintenance and updating of relevant information is therefore essential, as is the imposition of top-down control over incoming information and executive functions (Robbins, 2005). The activity of systems of the brainstem, which modulate processing in their terminal fields in diverse forebrain areas including the cortex, also appear to be under cortical monitoring (Roberts et al., 1994). This top-down control has been associated with the PFC (Fuster, 1989; Chao and Knight, 1995; Miller and Cohen, 2001). A crucial function of the PFC in response selection emerges in situations requiring the selection of rapid responses to novel, often stressful situations; then the 'supervisory attentional system' of Shallice and Norman (Shallice, 1982) becomes especially important, for example by adding more 'weight' to particular representations. Some such situations are changes in reward-error feedback (e.g. see Wisconsin Card Sort Test below), changes in background distractors or instructions (e.g. contextual control; Cohen et al., 1999), dual-task control and attentional conflict (e.g. Stroop interference).

Shallice and Norman's model attributed to the PFC the role of a 'supervisory attentional system'. The ventromedial orbitofrontal cortex (OFC) was subsequently implicated in emotional decision making (Damasio, 1998). This promoted speculations about how these PFC regions, with their limbic connectivity, interact with dorsolateral PFC regions in the control of cognition and behaviour. Investigation, largely using functional brain imaging, focused on the hypothesis that parts of the human medial cortex and OFC mediate 'reward' or 'goal' representations (O'Doherty et al., 2001). This view had to integrate accumulating evidence involving specified subcortical circuitry, notably DA-dependent functions of the nucleus accumbens, in the mediation of reward processes (Robbins and Everitt, 1992). This led to the recognition of the PFC as a nodal part of 'loop' circuitries, involving connections between the OFC, other limbic structures, the nucleus accumbens, mediodorsal thalamus and ventral pallidum. Such neuroanatomical loops link with other sectors of the PFC and functionally related regions of the striatum in a cascading series of serial as

well as parallel circuitries (Alexander et al., 1986; Haber et al., 2000). Functionally, these cortico-striatal loops can be understood as incorporating mechanisms for the optimal selection of goals and responses, and for the optimal preparation of appropriate response outputs (Robbins, 2007).

Neurochemical modulation of the PFC in attention

The functioning of cortico-striatal loops is influenced by a number of ascending neurotransmitter systems, notably the catecholamines (DA and noradrenaline), the indoleamine serotonin (5-HT) and acetylcholine (ACh) (Robbins, 2000). It is also likely that descending influences from the PFC may, to some extent, regulate these neurochemical systems (Amat et al., 2005) which are implicated in stress, arousal and mood as well as in reward processes (Robbins and Everitt, 1992; Arnsten and Robbins, 2002). These neurotransmitter systems are of fundamental importance to the aetiology of the neuropsychiatric conditions mentioned earlier, which share the core deficit of failure to regulate behaviour adequately in response to changing environmental demands.

As would be expected given the plethora of diffuse ascending inputs from the major monoaminergic and cholinergic neurotransmitter systems, the PFC needs to be highly sensitive to neurochemical state. Furthermore, it is now clear that the different functions of these ascending neurotransmitter systems need to be studied not only in general terms, but also when they project to a common substrate such as the PFC (Robbins, 2005), where they act in a neuromodulatory rather than in an 'on-off' manner. There is considerable evidence that the effects of pharmacological manipulations of many of these systems on tests of attention and memory can effectively be described by the characteristic inverted U-shaped curve. Thus, a specific manipulation may lead to improvement when superimposed on low baseline performance (e.g. due to fatigue or aging), whereas higher baseline performance may conceal such improvement or even show deterioration upon the same manipulation (Robbins, 2005).

Phasic activity in some of the neuromodulatory systems, especially the mesolimbic DA pathway,

has been implicated in the mechanisms of learning (Schultz and Dickinson, 2000). Their tonic levels of activity can be understood as representing different states (e.g. arousal, fatigue or mood). Tasks requiring executive control may be optimally performed in different states (Robbins, 2000). Executive control encompasses mechanisms serving to optimise behavioural and cognitive output and includes the regulation of input (e.g. over posterior cortical processing), output (e.g. via the basal ganglia and the associated cortico-striatal loops) and also the activity of the ascending neuromodulatory systems (Robbins, 2007).

Indexing attentional deficits in humans

Analysis of attentional deficits in disorders presenting prefrontal involvement has relied on a number of neuropsychological instruments. For example, sustained attention or vigilance has traditionally been examined through the continuous performance test (CPT; Rosvold et al., 1956; Parasuraman and Davies, 1977). In its original form the CPT requires sustained monitoring of sparse, unpredictable targets (e.g., letters) presented amongst distractors; performance deterioration over time is taken to reflect a vigilance decrement. Subsequent CPT modifications provide measures of visuo-spatial attention by requiring the subject continually to monitor the location of a brief visual target randomly occurring in one of the several spatial locations. Working memory can also be indexed by requiring responses only when the target is preceded by another stimulus.

A test broadly used in the investigation of the role of the PFC in cognitive flexibility is the Wisconsin Card Sort Test (WCST), which assesses deficits in attentional shifting. Initially, the test requires matching new stimuli to compound stimulus exemplars, following a constant rule or perceptual dimension. Thereafter, a category shift is required, that is the subject is required to start responding to a new rule, switching attention to a new perceptual dimension. Neuroimaging data confirm that completing the task primarily involves activation of the dorsolateral PFC. Finally, impaired behavioural inhibition, which is another common feature of neuropsychiatric

disorders such as Parkinson's, schizophrenia and most notably, ADHD has been studied by 'go-no go' procedures, such as the stop-signal reaction time (SSRT) task. It has even been argued that the form of inhibition represented by the SSRT is the only indisputable form of behavioural inhibition (MacLeod et al., 2003). In this task subjects are required to make fast responses on 'go' trials in a choice reaction time procedure, but to inhibit responding on signalled 'no-go' trials. The stop-signal occurs at different delays after the onset of the response process, thereby progressively taxing a subject's ability to impose response suppression.

Performance of patients with disorders involving attentional deficits (such as schizophrenia or ADHD) on neuropsychological instruments as those mentioned above is characterised by very high inter- and intra-individual variation. This variability, in addition to the multivariate nature of the attentional process, makes the task of exploring causal relations between neuropsychological data and the underlying neural substrate of attentional impairments extremely difficult, in spite of the armoury of neuroimaging techniques now available. This problem becomes especially acute when such causal relationships address neurochemical modulation, as systemic or localised infusion of selective pharmacological agents is not feasible in patients or healthy volunteers. Methods of pharmacological manipulation of neurotransmitter systems available for research in humans, such as tryptophan depletion (Rogers et al., 1999) have produced useful but essentially limited results. Studies on gene polymorphisms (Mattay et al., 2003) also have increasing potential. However, in order to ascertain neuro-anatomical and neurochemical specificity of experimental interventions, it is necessary to resort to the use of experimental animal models. This endeavour has been facilitated by the current availability of comparable cross-species tests of cognitive function. These enable the identification of common neural substrates that subserve similar functions across species, increasing the likelihood that the same cognitive functions are being studied in each species.

In this chapter, the neural substrates and the neuromodulation of basic operations such as

vigilance, set-shifting and executive control are surveyed, with a focus on the comparative functions of the DA and 5-HT systems and their interaction in the cognitive control exerted by the PFC. The survey is based on evidence from experimental animals and humans. It encompasses data generated by three different experimental conditions, each of which centres on a specific aspect of the attentional function (although of course not to the exclusion of other aspects). The three paradigms examined offer both animal and human variants.

The first paradigm is the five-choice serial reaction time task (5CSRTT) which provides a direct measure of sustained attention and bears good analogy to the CPT, a traditional index of human vigilance. The second paradigm is attentional set-shifting, which has been used to decompose the types of processes engaged by tests of attentional flexibility such as the WCST. The third paradigm is stop-signal inhibition, which models certain components of executive control.

In each case, the types of operation that are measured by the given paradigm and their neural correlates will be defined. Then, the role of the ascending dopaminergic and serotonergic systems in the neurochemical modulation of its behavioural output will be examined, with reference to clinical implications for neurological and neuropsychiatric disorders.

The five-choice serial reaction time task (5CSRTT)

5CSRTT, an animal test widely used with rodents, provides substantial validity as a direct measure of attention and bears good analogy to the CPT. The paradigm indexes different components of attention, so that the effects of various pharmacological treatments on these different attentional processes may be compared or contrasted. The 5CSRTT (Robbins et al., 1993; Robbins, 2002) is conducted in an operant chamber equipped with an arc of nine holes, four of which are occluded and five exposed. Each trial is initiated by the rat pushing open the food magazine door. This response is followed by a fixed 5-s intertrial interval (ITI), after which a 0.5 s light stimulus is presented

randomly in one of the five exposed holes. A nose-poke, within a 5-s hold period, in the hole where the light appeared is rewarded, while wrong responses are typically not punished.

Optimal performance on this apparently simple task requires the integration of several cognitive processes. Sustained attention to the goal area for the duration of the ITI is required in order not to miss the target, while divided attention across all five exposed holes is essential in order to scan the entire visual array. Good attentional performance is reflected by high response accuracy (a high number of correct target detections with a minimum of wrong responses), accompanied by few omissions and relatively fast response latencies. Generally speaking, deficient attention would result in low response accuracy. The likelihood that other factors, such as sensory, motor or motivational processes, also affect response accuracy can be assessed on the basis of the overall response profile on the task (Robbins, 2002; Chudasama and Robbins, 2003). Motivation can be indexed through the latency to collect reward; errors of omission with no change in reward collection latency indicate gross attentional impairments; while a concurrent reward collection latency increase suggests motivational or motor involvement. Changes in response latency without concurrent increase in reward collection latency possibly tap decisional processes. Finally, response inhibitory control (executive functioning) can also be assessed: the measure of premature responses during the ITI in anticipation of the visual target provides an index of impulsivity; while the inhibitory deficit of perseveration is also accessible through the measure of repeated responding at the holes, offering a putative index of compulsivity. Manipulations of task difficulty can be harnessed to explore the nature of any processing deficits. For example, response selection mechanisms can be excluded through use of a one-choice version, while a more robust assessment of sustained attention can be obtained by increasing the length of the ITI. Finally, sensory deficits can be examined by varying the intensity of the visual stimuli (Robbins, 2002). Thus, the 5CSRTT is capable of measuring several different types of performances, which include aspects of attention

and impulse control. The task is also capable of dissociating performance elements which usually co-vary, although they probably rely on processes that are under the control of different neural mechanisms.

It has been proposed that the 5CSRTT is particularly suited for testing attentional dysfunction in schizophrenia (Chudasama and Robbins, 2004). Several popular models of schizophrenic symptomatology are based on treatment with glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine (Steinpreis, 1996). The 5CSRTT appears to be sensitive to such psychotomimetic agents: systemic administration of PCP reduces choice accuracy, concurrently increasing premature and perseverative responding (Jin et al., 1997).

Effects of fronto-striatal lesions on the 5CSRTT

Attentional deficits accompanying schizophrenia have been consistently associated with frontal dysfunction, possibly as a result of dysfunction in the neural circuitry linking the PFC with the thalamus, cerebellum and possibly the basal ganglia. The hippocampus has also been implicated (see Introduction). This hypothesis can be readily investigated in rats by means of excitotoxic lesions to circumscribed areas of the PFC by means of the 5CSRTT.

There has been no consistent evidence for any hippocampal involvement in the 5CSRTT (Kirkby and Higgins, 1998). In contrast, medial PFC lesions involving the dorsal anterior cingulate cortex, medial prefrontal cortex and ventral infralimbic cortex reduce response accuracy, retard response latencies and increase perseverative responding (Muir et al., 1996). Selective lesions to these sub-regions in the rat demonstrated that they have quite specific functions, which must be coordinated to sustain optimal performance in the 5CSRTT. Specifically, accuracy impairments emerged only after dorsal anterior cingulate cortex lesions (Passetti et al., 2002; Chudasama et al., 2003); medial prefrontal or orbitofrontal cortical lesions produced selective increases in perseverative responding (Chudasama and Muir, 2001; Passetti et al., 2002) while, in contrast, lesions to

the ventral infralimbic cortex produced selective increases in premature responses (Chudasama et al., 2003). Evidence that the 5CSRTT engages fronto-striatal systems comes from the observation that bilateral lesions of the medial PFC or dorsal striatum result in deficits reproducible by the combination of unilateral medial prefrontal cortical and contralateral dorsal striatal lesion (Christakou et al., 2001). On the basis of neuro-anatomical substrate, the 5CSRTT therefore appears to be most appropriate for modelling those aspects of cognitive dysfunction in schizophrenia which are thought to depend on 'fronto-executive' processes.

Neurochemical modulation of the 5CSRTT

Several drugs of established therapeutic value in schizophrenia, in fact most atypical antipsychotic drugs such as clozapine or reserpine, are thought to exert their actions on 5-HT as well as on DA receptors. These agents appear preferentially to increase DA release in the medial prefrontal cortex (mPFC) (Meltzer et al., 1989; Moghaddam and Bunney, 1990; Kuroki et al., 1999). Experimental evidence suggests that discrete behavioural elements in the 5CSRTT may be differentially regulated by dopaminergic and serotonergic projections to the PFC (Dalley et al., 2002a, b; Winstanley et al., 2003).

Effects of dopaminergic manipulations

Subcortical DA systems. Subcortical manipulations of the DA systems have produced performance deficits on the 5CSRTT that are mainly expressed in terms of effects on the speed and probability of responding (Cole and Robbins, 1989; Baunez and Robbins, 1999).

The D2 receptor system. Systemic treatment with preferential D2 receptor antagonists such as sulpiride produced accuracy deficits at certain doses (Harrison et al., 1997). As DA D2 receptors are found in much higher numbers in subcortical loci such as the striatum rather than in the PFC, this effect is possibly consistent with effects of

dorsal striatal DA depletion (Baunez and Robbins, 1999). Intriguingly, however, systemic administration of sulpiride, which impaired performance in control animals, actually alleviated a response accuracy deficit noted in animals with mPFC lesions (Passetti et al., 2003). It can be hypothesised that the accuracy deficit noted in this study resulted from lesion-induced over-activity of subcortical dopaminergic systems, an explanation consistent with the lack of any effect of intra-mPFC infusions of sulpiride on the 5CSRTT (Granon et al., 2000).

The prefrontal D1 receptor system. Direct intra-mPFC infusions of a DA D1 receptor agonist (SKF 38393) significantly enhanced response accuracy in animals with low baseline accuracy but had no effect on animals with higher baseline performance. Conversely, intra-mPFC D1 antagonist (SCH 23390) infusions had no effect on animals with low baseline accuracy but reduced accuracy in animals with high baseline performance (Granon et al., 2000). This pattern suggests that the prefrontal D1 receptor system might normally be engaged to attain optimal task performance. The data also suggest that, under certain test conditions, it is feasible to enhance attentional performance in normal rats with a D1 receptor agonist and provides additional support for the efficacious use of D1 agonists in aged monkeys (Arnsten, 1997) or monkeys treated chronically with typical antipsychotic drugs which block D2 receptors concurrently down-regulating frontal D1 receptors (Lidow and Goldman-Rakic, 1994; Florijn et al., 1997; Lidow et al., 1997, 1998; Castner et al., 2000).

Taken together, these data indicate that dopaminergic projections to the rat mPFC have specific functions in modulating response accuracy in the 5CSRTT, while other aspects of performance such as response vigour or speed may be influenced by subcortical DA systems (Cole and Robbins, 1989).

Effects of serotonergic manipulations

As the dopaminergic system, the serotonergic system, the 5-HT_{1A} and 5-HT_{2A} receptors in

particular, is affected by atypical antipsychotics like clozapine (Meltzer, 1999; Millan, 2000; Winstanley et al., 2003). The serotonergic system, as a whole, has been strongly implicated in the regulation of impulsivity (Linnoila et al., 1983; Soubri , 1986).

The 5CSRTT is demonstrably sensitive to serotonergic manipulations. Global, 5,7-dihydroxytryptamine (5,7-DHT) lesion-induced 5-HT depletion consistently appears to spare response accuracy while it increases impulsivity as reflected by increased premature responding and decreased omissions as well as correct response latency (Harrison et al., 1997; Koskinen et al., 2000; Winstanley et al., 2003, 2004). However, systemic administration of the 5-HT1A receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT), which also decreases 5-HT release (Bonvento et al., 1992; Haj s et al., 1999; Celada et al., 2001), does not affect impulsive responding and improves attentional performance (Winstanley et al., 2003). At higher doses the selective 5-HT1A receptor agonist 8-OH-DPAT reportedly increased impulsivity, possibly by activating presynaptic 5-HT1A receptors (Carli and Samanin, 2000). There is an incongruence, then, between the effects of chronic lesion-induced global 5-HT decreases and acute global decreases as those affected by systemic administration of a 5-HT1A receptor agonist.

The apparent inconsistency is compounded by the observation that systemic and intra-PFC administration of the 5-HT2A receptor antagonist M100907 decreases impulsive responding (Winstanley et al., 2003). Moreover, infusions of M100907 in the mPFC counteracted the loss of executive control (impulsivity induced by the competitive NMDA receptor antagonist 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid, CPP), while 8-OH-DPAT decreased compulsive perseveration (Carli et al., 2006). Thus, an antagonist of the 5-HT system effectively produces effects opposite to those of global decrease in 5-HT transmission. This paradox, along with the observation that DOI, a 5-HT2A/2C agonist does increase premature responding, probably through activation of the 5-HT2A receptor (Koskinen et al., 2000), suggests dissociable behavioural contribution of 5-HT receptor subtypes in the 5CSRTT. Indeed, evidence suggests that the 5-HT2A and 5-HT2C receptors

have opposing neurochemical effects. 5-HT2C receptor activation inhibits, whereas 5-HT2A activation enhances DA release (Millan et al., 1998; Di Matteo et al., 2000, 2001). Antagonism of 5-HT2C and 5-HT2A receptors has opposite effects on some behavioural effects of cocaine (Fletcher et al., 2002). Furthermore, it has been demonstrated that 5-HT2C and 5-HT2A receptors also have contrasting and dissociable behavioural contribution on impulsivity in the 5CSRTT. The selective 5-HT2C antagonist SB 242084 increases premature responding and decreases correct response latency (Higgins et al., 2003; Winstanley et al., 2004). When the antagonist was administered to 5,7-DHT-lesioned animals, the increase in premature responding emerged over and above the similar effects of the 5,7-DHT lesion (Winstanley et al., 2004; Fig. 1A2). In contrast, the selective 5-HT2A antagonist M100907 had no effect on response latency and actually reduced premature responding (Fig. 1A1). This effect was abolished by 5,7-DHT lesions (Winstanley et al., 2004). This dissociation challenges the hypothesis that general decreases in 5-HT neurotransmission increase impulsivity. Furthermore, the fact that antagonism of the 5-HT2C receptor produces a behavioural profile closer to 5,7-DHT lesions than any other receptor so far tested including the 5-HT2A receptor, suggests that the 5-HT2C receptor is central in the serotonergic regulation of behavioural inhibition.

Compulsivity, another form of motor disinhibition is indexed by the 5CSRTT via perseverative responding. Winstanley et al. (2004) demonstrated that 5,7-DHT lesions increased perseverative as well as impulsive responding, a finding consistent with increased perseverative errors during reversal in the marmoset after localised 5-HT depletion within the PFC (Clarke et al., 2004) and after OFC damage (Jones and Mishkin, 1972; Rogers et al., 1999; Schoenbaum et al., 2002; Chudasama et al., 2003; Chudasama and Robbins, 2004). Neither 5-HT2A antagonism (M100907) nor 5-HT2C antagonism (SB 242084) appear to affect perseverative responses (Higgins et al., 2003; Winstanley et al., 2003, 2004). These data suggest that different kinds of motor disinhibition differ in their neurobiological bases, as impulsivity and compulsivity appear to be differentially regulated by the 5-HT system.

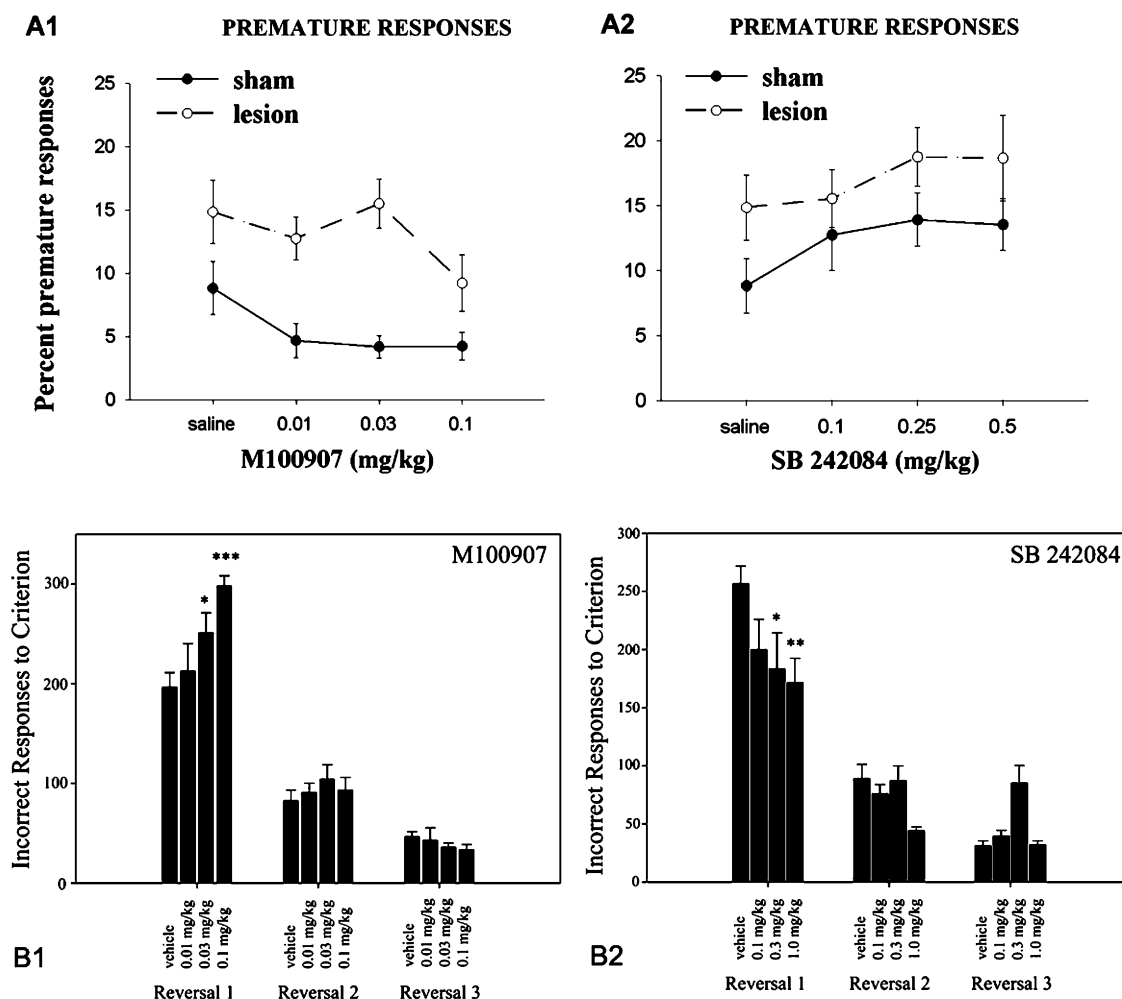


Fig. 1. Effects of M100907 (A1) and SB 242084 (A2) on the percentage of premature responses performed during the five-choice serial reaction time task (5CSRT) in ICV 5,7-dihydroxytryptamine (5,7-DHT)-lesioned animals and sham-operated controls. (Adapted with permission from Winstanley et al., 2004.) Effects of M100907 (B1) and SB 242084 (B2) on perseverative and learning errors performed during spatial reversal learning. (Adapted with permission from Boulougouris et al., 2007b.)

Taken together, the available evidence suggests that serotonergic modulation in the mPFC can increase attentional selectivity and decrease impulsivity via 5-HT_{1A} and 5-HT_{2A} receptors.

DA–5-HT interaction on the regulation of 5CSRTT performance

5,7-DHT lesions increase the number of premature responses and reduce correct response latency in

the 5CSRTT. Administration of amphetamine causes a similar pattern of behavioural effects (Cole and Robbins, 1987; Harrison et al., 1997). This amphetamine-induced increase in impulsivity is attenuated by serotonergic lesions (Harrison et al., 1997) and is dependent on the ability of amphetamine to increase DA release in the nucleus accumbens (Cole and Robbins, 1987, 1989). In contrast, the D1 receptor antagonist SCH 23390 decreases premature responding and reduces the increased impulsivity produced by 5,7-DHT

lesions (Harrison et al., 1997). These data implicate interactions between the 5-HT and DA system in regulating this form of impulsive behaviour.

During performance of a simplified version of the 5CSRT, in vivo microdialysis showed a marked increase of DA levels in the mPFC and a higher DA turnover was observed in the frontal cortex of more impulsive animals' post-mortem (Dalley et al., 2002a, b). It is possible that M100907 and SB 242084 may exert their opposite effects on impulsivity in this task via their contrasting modulation of the dopaminergic system. 5-HT_{2C} receptor antagonism increases basal levels of DA and noradrenaline (NA) efflux (Millan et al., 1998; Di Matteo et al., 2000; Gobert et al., 2000) while, in contrast, 5-HT_{2A} antagonism does not affect levels of DA and NA (Gobert and Millan, 1999). The increase in impulsive behaviour by SB 242084 (Winstanley et al., 2004) might therefore be mediated by enhanced DA release triggered by SB 242084. In contrast, a decrease in task-related dopaminergic activation potentially caused by M100907 may account for the decrease in premature responding observed.

Clinical implications

Improved attentional performance on the 5CSRTT following 8-OH-DPAT and M100907 may be due to cortical ACh release mediated by dopaminergic and serotonergic interactions at 5-HT_{1A} and D1 receptors (Winstanley et al., 2003), given that systemic 8-OH-DPAT as well as systemic DA D1 agonists increase prefrontal ACh release (Day and Fibiger, 1993; Consolo et al., 1996; Steele et al., 1997). These interacting mechanisms may facilitate attentional and cognitive improvements via atypical antipsychotic treatment in schizophrenic patients.

The evidence at hand therefore suggests that serotonergic modulation in the mPFC can increase attentional selectivity and decrease impulsivity via 5-HT_{1A} and 5-HT_{2A} receptors. These findings bear clinical relevance, given that some atypical antipsychotics have 5-HT_{2A} receptor antagonist actions that may potentially contribute to a pro-cognitive effect in schizophrenia (Meltzer, 1999).

Attentional set-shifting

Tests such as the WCST which index cognitive flexibility, in fact address several similar yet distinct forms of attentional shifts. For example, if we consider discrimination learning based on compound stimuli involving two perceptual dimensions (e.g. shapes and lines), where exemplars of these dimensions occur in combination with one another on successive trials, one exemplar of one particular dimension being correct (e.g. vertical but not skewed line correct), then (1) when the relevant stimulus dimension (i.e. lines) stays constant but novel stimuli are used (e.g. straight but not curly line correct), this is an intradimensional (ID) shift; (2) when an exemplar from the previously irrelevant dimension (shapes) becomes correct (square but not triangle) then an extra-dimensional (ED) shift is demanded; finally (3) when the stimuli remain the same, but the previously correct exemplar is now incorrect (triangle but not square) then we refer to reversal learning, a shift which can occur either at the compound discrimination learning stage or after the ID- or ED-shift.

Different tests of attentional flexibility involving ID–ED shifts and reversal are available for humans, non-human primates and rodents. Such procedures by necessity engage other processes besides switching attention (e.g. ability to utilise feedback denoting that a shift is necessary, ability to overcome ‘learned irrelevance’ of a previously non-operative perceptual dimension). However, the precise nature of any failure to make a required shift can be further analysed (Owen et al., 1993).

Effects of fronto-striatal lesions on set-shifting and reversal

Research on the neural substrate of attentional shifting has demonstrated that the apparently similar switching requirements of ID- and ED-shift are in fact mediated by different regions of the PFC (Dias et al., 1996, 1997; Robbins, 1998). Marmosets with lateral PFC lesions were impaired when an ED-shift (from ‘shapes’ to ‘lines’, a category shift, in terms of the WCST) was required. However, they were unimpaired in

reversal learning, which suggests that their ED-shift deficit was not simply a failure to detect altered feedback. When marmosets with lesions to the OFC were tested, a double dissociation was noted: these animals had no deficit on the ED-shift, but were impaired in reversal learning.

The lateral PFC–OFC functional dissociation has also been demonstrated in rats (Brown and Bowman, 2002), supporting the existence of neuroanatomical homologies between rodent and primate PFC regions (Preuss, 1995; Brown and Bowman, 2002). Lateral OFC lesions in the rat produce impairments in reversal learning (Schoenbaum et al., 2002; Chudasama and Robbins, 2003; Boulougouris et al., 2007a). A similar involvement of the rat medial PFC and the primate lateral PFC in ED-shifting might also be expected on the grounds of the putative homology of these regions, although this is more controversial (Brown and Bowman, 2002).

Translating these basic findings to humans proved difficult, as reversal learning is much easier for humans than ED-shifting. Nevertheless, relatively selective reversal deficits have been shown in patients with frontal-variant fronto-temporal dementia, for whom hypoperfusion initially occurs in the OFC (Rahman et al., 1999). This finding, which suggests that the human OFC also mediates aspects of reversal learning, has been corroborated by other neuropsychological studies (Fellows and Farah, 2003; O'Doherty et al., 2003; Hornak et al., 2004).

However, data relating to specific PFC regions with ED-shifting are sparse. Patients with PFC damage of varied aetiology, but in whom the OFC had been spared, showed maximum deficit in ED-shifting, while reversals were not significantly affected (Owen et al., 1991).

Exploration of the neuroanatomical substrate of reversal learning and shifting in a functional imaging context (positron emission tomography, PET; Rogers et al., 2000) was initially unsuccessful in showing selective OFC activation, perhaps due to the nature of the task used. However, the Rogers et al. (2000) study demonstrated activation of the ventromedial caudate nucleus in the contrast between ID-shift and reversal, suggesting that reversal is mediated in part by a cortico-striatal loop: this would include the OFC, given the

anatomical connectivity existing between these regions. In the same study, contrast between ED- and ID-shifting showed activity in the rostral and dorsolateral PFC. A recent study (Hampshire and Owen, 2006) employing event-related functional magnetic resonance imaging (fMRI) and methods allowing better resolution of activity within the OFC, demonstrated that reversal was associated with blood oxygen level-dependent (BOLD) signals in the OFC, with concurrent reduction in medial PFC activation. ED-shifting was most obviously associated with ventrolateral PFC activity. Although the dorsolateral PFC was not specifically associated with responding at any one stage, it was active during most of the task, which suggests an overall role in strategic processes contributing to problem solution.

In conclusion, if the lateral PFC region in the marmoset corresponds to the ventrolateral PFC highlighted in the Hampshire and Owen (2006) study, then there is concordance between the marmoset lesion data and human functional neuroimaging results. The lesion data suggest that both the lateral and OFC regions of the marmoset PFC are active during discrimination learning, but have different functions in behavioural plasticity. The lateral PFC appears to control the shifting of responding between entire, abstract perceptual dimensions (e.g. 'shape' vs. 'line'), whereas the OFC mediates the shifting of responding between simple concrete features with specific associations with reward. Over and above showing functional specialisation of PFC regions, these data imply a hierarchical organisation of function between lateral and OFC regions, analogous to other proposed hierarchical relations between PFC areas (Petrides, 1998; Koechlin et al., 2003). Indeed, an influential theory of discrimination learning holds that discrimination learning proceeds hierarchically (Sutherland and Mackintosh, 1971). The data suggest that the different stages of discrimination learning correspond to different functions mediated by the lateral PFC and the OFC.

The behavioural outcome of both lateral PFC and OFC lesions is perseverative responding, either to previous exemplars or to dimensions in the face of non-reward, both deficits reflecting defective behavioural inhibition. The Dias et al.

(1996, 1997) findings therefore suggest that both the lateral PFC and the OFC contribute to inhibitory functions in response selection. Thus, contrary to earlier opinions, response inhibitory functions appear to be distributed widely within the PFC, in analogy to Goldman-Rakic's view that working memory is organised on a modular basis within the PFC, subsuming processes of inhibition and selection, as well as holding stimuli online.

Neurochemical modulation of attentional set-shifting and reversal

As mentioned earlier, hypoactivity of the mesocortical DA projection has been implicated in clinical disorders such as schizophrenia and ADHD, as well as in working memory dysfunction (Goldman-Rakic, 1998). Consequently, the effects of dopaminergic manipulations on attentional shifting have been examined. The established contribution of prefrontal 5-HT in executive control also led to exploration of the serotonergic contribution to attentional flexibility.

Effects of dopaminergic manipulations

Profound DA depletion from the entire PFC in the marmoset actually enhanced rather than impairing ED-shifting (Roberts et al., 1994; Fig. 2). This unexpected finding was later attributed to an initial failure of the monkeys to form stable attentional sets, since DA depletion profoundly impaired serial ID-shifting (Crofts et al., 2001; Fig. 2), which normally leads to the establishment of an attentional set. Dopamine depletion had no other effects on discrimination acquisition or simple or serial reversal learning (Roberts et al., 1994; Clarke et al., 2007; Fig. 2). In the rat, pharmacological inhibition of catechol-*o*-methyltransferase (COMT: postulated to have a selective effect on PFC dopamine) by tolcapone, resulted in improvements in ED-shifting, possibly as a consequence of enhanced PFC dopamine activity (Tunbridge et al., 2004).

Selective orbitofrontal DA depletion in the marmoset was without effect in either acquisition of visual discriminations or reversal learning (Clarke et al., 2007). Similarly, selective striatal

DA depletion in the marmoset caudate nucleus had no effect on discrimination learning, reversal, ID- or initial ED-shifting, though it reduced distractibility in the ID-ED-shift task (Collins et al., 1998; Crofts et al., 2001). However, a deficit was observed when, at the end of the series, an ED-shift back to the previously reinforced dimension was introduced. The finding suggests that, while the striatum and its dopaminergic innervation may not be involved in the formation of new sets, they may be important in the mediation of shifts between already established sets. Additionally, DA depletion of the rodent dorsomedial striatum selectively impaired reversal of odour discriminations, though discrimination acquisition was intact (O'Neill and Brown, 2007). This suggests that dopaminergic transmission in the dorsomedial striatum contributes to reversal.

In humans, an fMRI study on normal volunteers, revealed activations only in the PFC following rule alternation, while reversal produced activations in both the PFC and striatum (Cools et al., 2004). Also, patients with striatal lesions (though mainly comprising lesions of the putamen and not the caudate nucleus) were unimpaired in responding to rules but exhibited problems alternating between objects (Cools et al., 2006). Finally, a probabilistic reversal task activated not only regions of the OFC, medial PFC and inferior frontal cortex (IFC), but also the ventral striatum (Cools et al., 2002). These data suggest that the PFC controls set shifting, while both the PFC and the striatum are involved in the reversal. More direct assessment of the relative contributions of cortical and striatal DA in the two types of attentional shifting are difficult in humans, as it is hard to manipulate the mesocortical DA system selectively. Nevertheless, studies on polymorphism for a gene controlling COMT showed some deficits in WCST performance, suggesting a difficulty in ED-shifting rather than in ID-shifting (Mattay et al., 2003). In normal volunteers, a D2 receptor antagonist (sulpiride) produced weak and inconsistent effects only on set shifting latency (Mehta et al., 2004). Finally, a study on Parkinson's patients (Lewis et al., 2005) showed an impairment in set-shifting which was unaffected by L-DOPA, while a parallel working memory deficit was ameliorated by L-DOPA.

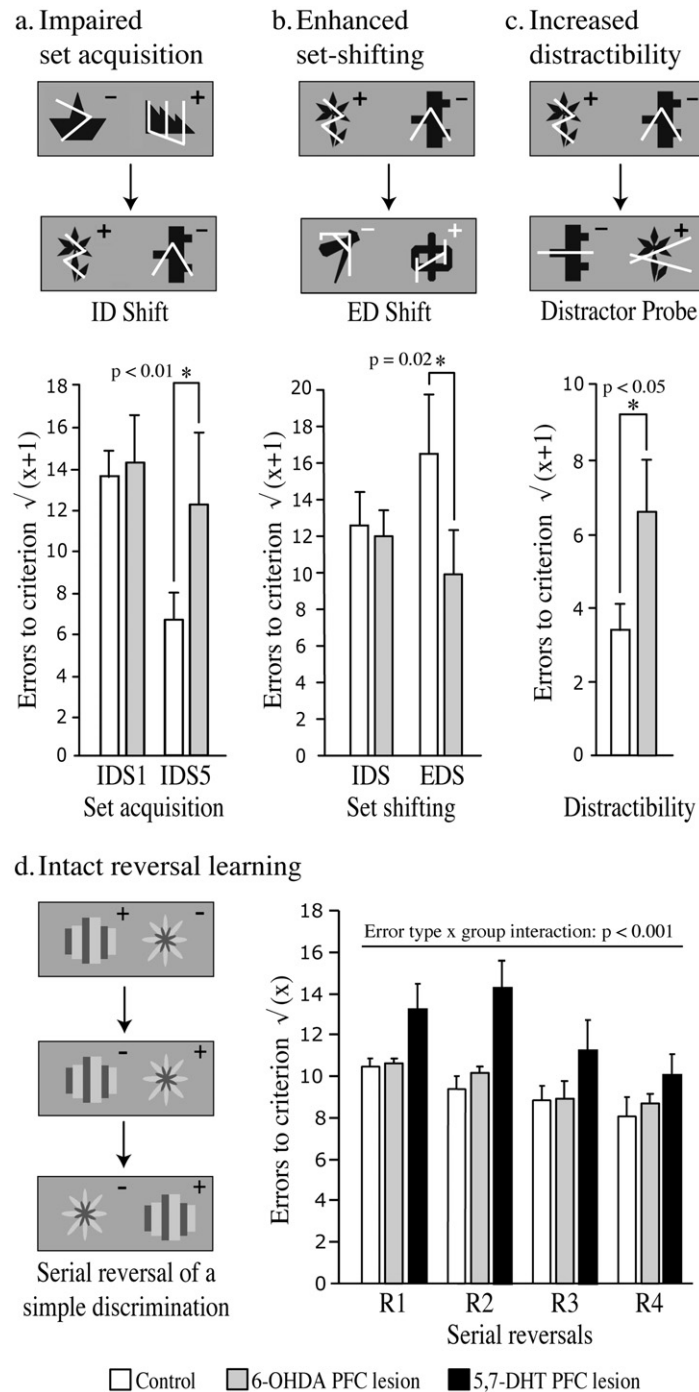


Fig. 2. The effects of 6-OHDA induced DA lesions of the marmoset PFC on the acquisition and shifting of attentional sets and on reversal learning. Examples of two discriminations in which the same dimension remains relevant, commonly called an IDS, are shown in (a); a discrimination requiring a shift of attentional set, called an EDS, is depicted in (b); a distractor probe test, shown in (c), in which the exemplars from the irrelevant dimension of a previously learned discrimination are replaced by novel exemplars. The '+' and '-' signs in (a), (b) and (c) indicate, respectively, whether the stimuli were associated with reward or not. Black lettering indicates that shapes were the relevant dimension and white lettering that lines were the relevant dimension. (Adapted with permission from Robbins and Roberts, 2007.)

Although clinical and preclinical evidence suggests that deregulation within the dopaminergic systems is involved in behavioural flexibility and inhibition, the particular receptor mechanisms underlying these effects are still not well understood. Studies on the effects of D-amphetamine, which increases DA release in the striatum, have produced equivocal results of either impairment or facilitation of reversal performance (Weiner and Feldon, 1986; Idris et al., 2005). Involvement of the D2 receptor seems likely, at least in reversal: the D2 receptor antagonist haloperidol impairs reversal performance (Ridley et al., 1981), as does blockade of the D2 receptor gene in knockout mice (Kruzich and Grandy, 2004). Reversal is also compromised by the D2/D3 receptor antagonist raclopride (monkeys: Lee et al., 2007) and the D2/D3 receptor agonist quinpirole (rat: Boulougouris et al., unpublished observations). There is also some indication of DA D2/D3 receptor involvement in the modulation of set shifting. Floresco et al. (2006) showed that administration of the D2/D3 receptor antagonist eticlopride impaired animals' ability to adjust their behaviour to a conditional change of rule in a set-shifting task.

Overall, the data suggest a separation of function between PFC and striatum with respect to various forms of attentional flexibility. While mesocortical DA was initially implicated in set-shifting, subsequent studies argue against the participation of either prefrontal or striatal DA, at least in the mediation of the ED-shift. It is possible that the ED-shift depends on PFC interactions with other cortical regions, especially in the parietal and temporal cortices (Rogers et al., 2000; Hampshire and Owen, 2006). On the other hand, while there is consensus of a lack of effect of cortical DA on reversal learning, several lines of evidence implicate subcortical systems in its mediation. D2 receptors in the striatum would appear to be implicated.

Effects of serotonergic manipulations

In contrast to PFC dopamine depletion, selective 5-HT depletion in the marmoset had no effect on ED- or serial ID-shifting, but produced a large

deficit in reversal learning due to perseverative responding to the previously rewarded object (Clarke et al., 2004, 2005, 2007).

In human volunteers, transient depletion of central 5-HT by the tryptophan depletion technique produced effects on discrimination learning that were especially evident in reversal learning (Park et al., 1994). Another study (Rogers et al., 1999b) also reported that tryptophan depletion led to relatively selective effects on human reversal learning (but see also Talbot et al., 2006) with no effect on ED-shifting. Evers et al. (2005) showed that behavioural reversal was accompanied by significant signal change in the right ventrolateral and dorsomedial PFC of healthy volunteers performing a probabilistic reversal task. Tryptophan depletion enhanced reversal-related signal change in the dorsomedial PFC only, affecting the BOLD signal specifically associated with negative feedback. These data indicate that the 5-HT system has a modulatory role in reversal learning specifically.

On the receptor level, recent evidence suggests that different 5-HT receptor subtypes have distinct roles in the modulation of reversal learning. Boulougouris et al. (2007a) established a double dissociation in the role of 5-HT_{2C} and 5-HT_{2A} receptor subtypes in serial spatial reversal learning. Specifically, systemic administration of the 5-HT_{2C} receptor antagonist SB 242084 facilitated spatial reversal learning in a dose-dependent manner (Fig. 1B2). Selective intra-OFC infusions of SB 242084 also promoted reversal learning, whereas infusions in the mPFC or nucleus accumbens did not. The facilitation of reversal learning therefore appears to be mediated by 5-HT_{2C} receptors within the OFC (Boulougouris and Robbins, unpublished observation). In contrast, systemic treatment with the 5-HT_{2A} receptor antagonist M100907 dose-dependently impaired reversal learning, on the first reversal of the series in particular (Fig. 1B1). This deficit emerged as increased perseveration of the previously correct response, reproducing the effects observed after selective orbitofrontal 5,7-DHT lesions (Clarke et al., 2004, 2005, 2007) as well as orbitofrontal cortical lesions in rats and non-human primates (Dias et al., 1996; Chudasama and Robbins, 2003; Boulougouris et al., 2007a).

These findings are of considerable theoretical and clinical importance. At a theoretical level, the opposing effects of 5-HT_{2A} and 5-HT_{2C} antagonism on perseverative responding in spatial reversal learning task (increase and decrease, respectively) contrast with the also reverse effects of these agents on impulsive responding in the 5CSRTT (see section The five-choice serial reaction time task (5CSRTT); Fig. 1). Specifically, intra-PFC 5-HT_{2A} antagonism decreases impulsive responding (Winstanley et al., 2003) whereas 5-HT_{2C} antagonism increases it (Higgins et al., 2003; Winstanley et al., 2004). These observations are relevant to the concept of an impulsivity–compulsivity spectrum in obsessive–compulsive spectrum disorders (Hollander and Rosen, 2000). At a clinical level, these data also bear on the issue of whether 5-HT_{2C} receptor antagonists might be expected to be useful in the treatment of human obsessive–compulsive disorder (OCD).

DA–5-HT interaction on the regulation of set-shifting and reversal

In summary, 5-HT neurotransmission in the OFC contributes to the modulation of reversal learning, with distinct and contrasting roles of the 5-HT_{2A} and 5-HT_{2C} receptors in this modulation of reversal. No impact on the performance of tasks such as ED-shifting has been discerned so far.

In contrast, the mesocortical DA projection has been implicated in set-shifting, while there is consensus on its lack of involvement on reversal learning. However, the striatal dopaminergic innervation appears to contribute to the modulation of reversal learning and possibly in the mediation of shifts between already established sets. Therefore, although both the dopaminergic and serotonergic systems innervate the entire PFC, they appear to have differential impact in distinct regions, since manipulation of the two monoamine pathways has distinct effects on PFC-dependent mechanisms of cognitive flexibility.

With respect to the functional dissociation of the 5-HT_{2A} and 5-HT_{2C} receptor role in the modulation of reversal, it is possible that M100907 and SB

242084 exert their opposite effects on compulsivity in this task via their contrasting modulation of the dopaminergic system. As mentioned earlier (see section The five-choice serial reaction time task (5CSRTT)) 5-HT_{2C} receptor antagonism increases basal levels of DA and NA efflux (Millan et al., 1998; Di Matteo et al., 2000; Gobert et al., 2000) while, in contrast, 5-HT_{2A} antagonism does not affect levels of DA and NA (Gobert and Millan, 1999). The facilitation of reversal, implying minimal perseveration to a previously rewarded response (Boulougouris et al., 2007b) might be mediated by enhanced DA release triggered by SB 242084, while a decrease in task-related dopaminergic activation potentially caused by M100907 may account for the increased perseveration caused by the 5-HT_{2A} receptor antagonist.

Clinical implications

Patients with basal ganglia disorders, such as early Huntington's and Parkinson's diseases, show impairments in ED-shifting, suggesting some mediation by striatal structures. In late in-the-course Huntington's disease, impairments in simple reversal learning are prohibitive of attempts at examining ED-shifts. This pattern of initial deficits in ED-shifting followed by reversal learning deficits suggests a dorsal-to-ventral spread in pathology (Lange et al., 1995). In late in-the-course Parkinson's, performance in the early stages of ID- and ED-shift was remediated by L-DOPA, although there was no conclusive evidence on whether ED-shifting was affected (Lange et al., 1992). As mentioned earlier, the hypothesis that the ED-shift is DA-dependent now appears doubtful (Mehta et al., 1999, 2004; Cools et al., 2001; Lewis et al., 2005), raising the possibility that it may be modulated by PFC interactions with other cortical regions (Rogers et al., 2000; Hampshire and Owen, 2006). The deficits observed in ED-shifting in Parkinson's and Huntington's diseases may thus reflect extra-striatal pathology, possibly in the PFC.

Finally, the Boulougouris et al. (2007b) data suggest that 5-HT_{2C} receptor antagonists may be useful in relieving reversal deficits such as those

noted in Huntington's disease. In fact, they may deserve consideration as a means of controlling compulsivity in the context of obsessive-compulsive disorder.

The stop-signal reaction time task (SSRT)

The SSRT task has been used as a measure of behavioural inhibition in humans, non-human primates and rodents, and is ideally suited for translational study. The SSRT is a sophisticated 'go-no-go' task in which subjects are required to make speeded responses on 'go' trials in a choice reaction time procedure, but to inhibit responding on 'no-go' trials. These are approximately 25% of the total trials and are signalled by a succinct auditory stimulus. This stop-signal is programmed to occur at different delays following the imperative signal, thus occurring at different times after the onset of the response process. Therefore, the ability of a subject to impose response suppression can be progressively more taxed.

The response outcome of a stop trial is dependent on which response process will finish first: if it is the 'stop' process the response will be inhibited, if it is the 'go' process then the response will be performed. Consequently, trials in which the stop signal is presented late on in the response process are less likely to be inhibited than trials in which the stop signal is presented early in the response process. The central measure of the task is the speed of the response stopping process, that is the time taken by the subject to attend to, process and complete a response to the stop signal. The stop response has no physical outcome. The estimate of the end point of such a response is based on the theoretical framework provided by the 'horse-race model' (for details see Logan and Cowan, 1984), upon which the SSRT task is based.

The SSRT is particularly suited for testing executive aspects of attentional dysfunction. It has been argued, perhaps debatably, that the form of inhibition represented by the SSRT is the only indisputable form of behavioural inhibition (MacLeod et al., 2003).

Effects of fronto-striatal lesions on the SSRT

Both stopping and no-go impairments have been extensively associated with fronto-striatal dysfunction (Rubia et al., 2001; Robbins, 2007). Evidence comes from translational neuroanatomical studies which have highlighted regions of the frontal cortex and basal ganglia that are critical for response inhibition, and interplay between these regions may be necessary for attaining appropriate behavioural outcomes (Band and van Boxtel, 1999). Monkeys with lesions of the inferior convexity, a likely homologue of the human right inferior frontal gyrus, produced impairments in go/no-go performance (Iversen and Mishkin, 1970), while human subjects with frontal cortical damage were impaired in response inhibition (Drewe, 1975; Decary and Richer, 1995; Godefroy and Rousseaux, 1996). Recent neuroimaging studies have highlighted several cortical regions of interest with respect to both SSRT and go/no-go tasks. In particular, several studies report strong IFC activation in both stop and go/no-go tasks, thus underlining the importance of this region in behavioural inhibition (Aron et al., 2004).

In rats, the role of the PFC in SSRT control is not well understood. Recently, it has been shown that excitotoxic lesions of the rat OFC, but not the infralimbic or prelimbic cortex, slowed SSRT but had no significant effect on the go response (Eagle and Robbins, 2003; Eagle et al., 2007a). Although direct homology between the right IFC in humans and the ventral OFC in rats is not established, these regions are currently the only cortical regions, in the respective species, to be specifically implicated in the control of SSRT.

There has also been evidence for striatal involvement in the SSRT. In childhood and adolescent ADHD, subcortical structures have been shown to play an increased role in the processing of stop and no-go signals. Subjects with ADHD exhibited lower activation within the striatum than controls, while there was no difference in activation levels in the cortex between groups (Vaidya et al., 1998). There may be a reliance on subcortical structures in SSRT processing in younger subjects, with caudate activation in

adolescents that is not always seen in adult performance (Rubia et al., 1998, 2005b; Rubia and Smith, 2004; Nosarti et al., 2006). However, adult patients with basal ganglia lesions are impaired at stopping (Rieger et al., 2003).

Finally, the subthalamic nucleus (STN) is conventionally thought of as an output structure of the basal ganglia, acting as part of the potentially inhibitory indirect pathway within the cortico-striato-thalamic circuitry. Current interest in its function during the stopping process has led to a hypothesis that it links more directly with regions of the cortex involved in stopping, providing rapid information-processing during this form of inhibition. In human subjects, STN activation correlated with faster SSRTs (Aron and Poldrack, 2006) and STN activation on the SSRT task also correlated with activation of the right IFC. Additionally, SSRT deficits have been linked with abnormal STN function in Parkinson's disease (Gauget al., 2004) and stimulation within the STN, but not the surrounding structures, in these patients improved SSRT (van den Wildenberg et al., 2006). However, in the rat, lesions of the STN globally disrupted performance on the SSRT task, both when the stop signal was delayed, and when the stop signal was presented at the same time as the go signal, more strongly indicative of a generalised attentional or response selection (no-go-like) deficit following these lesions (Eagle et al., 2007a).

Neurochemical modulation of the SSRT

Effects of dopaminergic manipulations

As discussed previously, DA is strongly implicated in behavioural inhibition. It has been suggested that DA dynamically modulates the balance of go and no-go basal ganglia pathways during cognitive learning and performance (Frank et al., 2006). However, the effects Frank et al. (2006) define as increased inhibition may be construed as a negative modulation of the go pathway rather than as positive modulation of the no-go pathway and there is little evidence to support a role for either D1 or D2 receptors in no-go inhibition. Inase et al. (1997) investigated

the effect of the D1 and D2 receptor agonists (SKF38393-quinpirole) and antagonists (SCH23390-sulpiride) on single unit activity in the putamen of monkeys performing a go/no-go task. D1 and D2 receptor agents could modulate the activity of neurons in both go and no-go trials but there was no selective difference between go and no-go trials in the effectiveness of D1 or D2 manipulations. Additionally, in rats, the mixed D1/D2 antagonist, cis-flupenthixol, had no significant effect on no-delay (no-go) stop-trial accuracy (Eagle et al., personal communication), which again fails to support a role for D1 and D2 receptors in no-go inhibition.

Although D-amphetamine- and methylphenidate have been shown to induce speeding of SSRT, this may reflect action on striatally mediated DA function, as no firm evidence supports a role for DA in their action on the stopping process per se. Dopaminergic drugs can clearly increase impulsive behaviour on other tasks, for example delayed reward (Wade et al., 2000), but such drugs have little effect on stopping. Overtom et al. (2003) found no effect of L-DOPA on stopping. Although Fillmore et al. (2002) reported that cocaine users were impaired on SSRT compared with non-cocaine-using control subjects, which suggests dopaminergic involvement in SSRT, it was not possible to determine whether these differences predated or resulted from cocaine use.

The mixed D1/D2 receptor antagonist, cis-flupenthixol, had no effect either on stopping or on the SSRT-speeding effects of methylphenidate and modafinil at doses that significantly slowed the go response (Eagle et al., 2007b). While it is possible that methylphenidate or D-amphetamine might act via other DA receptors, there is no clear evidence to support a dopaminergic mechanism of SSRT control on the receptor level. Although polymorphisms in the DA receptor D4 (DRD4) gene in ADHD are thought to be critical for cognitive function, a comparison of ADHD children with or without at least one DRD4 7-repeat allele, found no difference in stopping behaviour, although there was a difference in GoRTs (Langley et al., 2004). Altogether, the evidence, at present, is against a direct role for DA in the stopping process.

Effects of serotonergic manipulations

As discussed previously, central 5-HT function is widely acknowledged as an important factor of behavioural inhibition and response control. Accumulating evidence implicates 5-HT on the modulation of no-go inhibition and stopping performance. Global 5-HT depletion in rodents following intracerebroventricular (i.c.v.) infusions of 5-7-DHT profoundly impaired the ability of rats to adequately inhibit responding to a no-go signal and also impaired the ability of pre-trained rats to subsequently respond correctly to a no-go signal (Harrison et al., 1999). This impairment was selective to an animal's ability to withhold responding following 5-HT depletion, without affecting other behavioural measures. Similarly, impaired acquisition of a go/no-go task has been reported after parachloroamphetamine administration (Masaki et al., 2006).

Neuroimaging studies in humans have implicated the OFC in relation to the effects of 5-HT on no-go inhibition. Acute tryptophan depletion has been reported to decrease right orbito-inferior prefrontal activation in fMRI during the no-go condition, although there was no significant alteration in inhibitory performance on the task (Rubia et al., 2005a). Moreover, fMRI data indicate that citalopram enhanced the response to the no-go condition in the medial orbitofrontal region (Del-Ben et al., 2005). Additionally, an fMRI investigation of healthy subjects' neural responses with or without the antidepressant mirtazapine during performance of a go/no-go task reported significant activation in the right dorsolateral PFC, middle frontal gyrus and OFC bilaterally, right anterior cingulate, right temporal and right parietal cortex and left occipital cortex and thalamus. Mirtazapine, however, enhanced activation in the right lateral OFC (Vollm et al., 2006). Treatment with *m*-chlorophenylpiperazine (mCPP; a non-specific 5-HT agonist) has also been shown to increase BOLD signal in the right OFC during go/no-go in healthy adults (Anderson et al., 2002).

Finally, there is evidence implicating the 5-HT_{2A} receptor in the no-go inhibition. A polymorphism in the promoter of the 5-HT_{2A} receptor gene has been proposed to underlie some forms of behavioural inhibition. Subjects with the

A-1438A allele of the 5-HT_{2A} receptor gene made more commission errors under the punishment–reward condition in a go/no-go task than those in the G-1438G group (Nomura and Nomura, 2006). The specific contribution of other 5-HT receptor subtypes in no-go responding is still unknown.

Although there is strong evidence on the role of 5-HT in no-go, there is no evidence regarding 5-HT role in the modulation of stopping performance. Depletion of brain 5-HT has relatively little effect on SSRT, even in subjects stratified according to 5-HT transporter polymorphism. Neither buspirone (a 5-HT_{1A} receptor agonist) nor citalopram (selective 5-HT reuptake inhibitor) had any effects on SSRT in healthy volunteers (Chamberlain et al., 2006a, b). Studies in rats corroborate the lack of effect with citalopram, additionally demonstrating that global (i.c.v.) 5, 7-DHT lesions have no effect on SSRT or any other behavioural measures (Eagle et al., personal communication). Moreover, 5-HT transporter knockout mice did not differ from wild type controls in the SSRT task (Hausknecht et al., 2006). Under these lines of reports and the use of SSRT in modelling impulsivity in juvenile and adult ADHD (Aron et al., 2003a, b), one might conclude that serotonergic agents do not seem to be useful for the treatment of this disorder where attentional deficits are a feature.

Conclusions

This survey provides an integrative account of the differential contributions of 5-HT and DA to specific aspects of attentional processes as they emerge from 'animal to human' approaches. Three tasks allowing translational study have been used to that purpose, to address three fundamental qualities of attention. (1) The 5CSRTT, an analogue of the human CPT, is designed to measure several attentional operations with an emphasis on sustained attention or vigilance. (2) Attentional set-shifting including reversal, intra- and extradi-mensional shifts, as the human WCST, tap attentional flexibility, that is the ability of humans and animals to develop and maintain higher-order rules and shift attention according to changing reward

Table 1. Neuropharmacology of the five-choice serial reaction time task (5CSRTT), intradimensional (ID)/extradimensional (ED) shift, reversal learning and serial reaction time task (SSRTT)

Task	Enhancement	Impairment
5CSRTT	Intra-mPFC D1 agonist (SKF 38393) Systemic D2 antagonists in mPFC-lesioned animals Intra-mPFC 5-HT _{2A} antagonist (M100907) Intra-mPFC 5-HT _{1A} agonist (8-OH-DPAT)	Systemic D2 antagonist (sulpiride) Intra-mPFC D1 antagonist (SCH 23390) Systemic D-amphetamine Global 5-HT depletion Systemic 5-HT _{2C} antagonist (SB242084)
ID-shift	Dopamine depletion	Dopamine depletion
ED-shift	D2/D3 antagonist eticlopride	Dorsomedial striatal dopamine depletion
Reversal	D2 antagonist (haloperidol) D2/D3 antagonist (raclopride) Systemic 5-HT _{2C} antagonist (SB 242084) Intra-OFC 5-HT _{2C} antagonism (SB 242084)	Systemic D2/D3 agonist (quinpirole) OFC serotonin depletion Systemic 5-HT _{2A} antagonist (M100907)
SSRTT	D-amphetamine	Cocaine; parachloroamphetamine

contingencies. (3) Finally, the SSRT addresses the issue of behavioural control by means of inhibition of activities which no longer serve environmental demands. Taken together, the findings detailed above highlight the specificity of influences that these neurotransmitter systems have on overall prefrontal executive control, acting to promote distinct components of prefrontal processing in a context-dependent manner (Table 1). Future directions must focus towards the definition of the specific aspects of attentional functions in which these neuromodulatory systems are acting to influence prefrontal processing. Of cardinal importance for the elucidation of the function of those neurotransmitters is their top-down regulation by the very system that they themselves modulate, that is the fronto-executive system.

Abbreviations

5,7-DHT	5,7-dihydroxytryptamine
5CSRTT	five-choice serial reaction time task
5-HT	5-hydroxytryptamine (serotonin)
8-OH-DPAT	8-hydroxy-2-(di- <i>n</i> -propylamino)-tetraline
ACh	acetylcholine
ADHD	attention deficit/hyperactivity disorder
BOLD	blood oxygen level-dependent
COMT	catechol- <i>o</i> -methyltransferase

CPP	3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid
CPT	continuous performance test
DA	dopamine
ED	extradimensional
fMRI	functional magnetic resonance imaging
i.c.v.	intra-cerebroventricular
ID	intradimensional
IFC	inferior frontal cortex
ITI	intertrial interval
mCPP	<i>m</i> -chlorophenylpiperazine
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NA	noradrenaline
NMDA	<i>N</i> -methyl-D-aspartate
OCD	obsessive-compulsive disorder
OFC	orbitofrontal cortex
PCP	phencyclidine
PET	positron emission tomography
PFC	prefrontal cortex
SSRT	stop-signal reaction time
STN	subthalamic nucleus
WCST	Wisconsin Card Sort Test

Acknowledgements

VB is supported by the Domestic Research Studentship, the Cambridge European Trusts, the Bakalas Foundation Scholarship and the Oon Khye Beng Ch'ia Tsio Studentship from the Downing College.

References

- Alexander, G.E., DeLong, M.R. and Strick, P.L. (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.*, 9: 357–381.
- Amat, J., Baratta, M.V., Paul, E., Bland, S.T., Watkins, L.R. and Maier, S.F. (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat. Neurosci.*, 8(3): 365–371.
- Anderson, I.M., Clark, L., Elliott, R., Kulkarni, B., Williams, S.R. and Deakin, J.F. (2002) 5-HT(2C) receptor activation by *m*-chlorophenylpiperazine detected in humans with fMRI. *Neuroreport*, 13(12): 1547–1551.
- Andreasen, N.C., Paradiso, S. and O'Leary, D.S. (1998) "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? *Schizophr. Bull.*, 24(2): 203–218.
- Arnsten, A.F. (1997) Catecholamine regulation of the prefrontal cortex. *J. Psychopharmacol.*, 11(2): 151–162.
- Arnsten, A.F.T. and Robbins, T.W. (2002) Neurochemical modulation of prefrontal cortical functions in humans and animals. In: Stuss D. and Knight R. (Eds.), *The Prefrontal Cortex*. Oxford University Press, New York, NY, pp. 51–84.
- Aron, A.R., Dowson, J.H., Sahakian, B.J. and Robbins, T.W. (2003a) Methylphenidate improves response inhibition in adults with attention-deficit/hyperactivity disorder. *Biol. Psychiatry*, 54(12): 1465–1468.
- Aron, A.R., Fletcher, P.C., Bullmore, E.T., Sahakian, B.J. and Robbins, T.W. (2003b) Stop-signal inhibition disrupted by damage to right inferior frontal gyrus in humans. *Nat. Neurosci.*, 6(2): 115–116.
- Aron, A.R. and Poldrack, R.A. (2006) Cortical and subcortical contributions to Stop signal response inhibition: role of the subthalamic nucleus. *J. Neurosci.*, 26(9): 2424–2433.
- Aron, A.R., Robbins, T.W. and Poldrack, R.A. (2004) Inhibition and the right inferior frontal cortex. *Trends Cogn. Sci.*, 8(4): 170–177.
- Aylward, E.H., Anderson, N.B., Bylsma, F.W., Wagster, M.V., Barta, P.E., Sherr, M., Feeney, J., Davis, A., Rosenblatt, A., Pearlson, G.D. and Ross, C.A. (1998) Frontal lobe volume in patients with Huntington's disease. *Neurology*, 50(1): 252–258.
- Band, G.P.H. and van Boxtel, G.J.M. (1999) Inhibitory motor control in stop paradigms: review and reinterpretation of neural mechanisms. *Acta Psychol.*, 101(2–3): 179–211.
- Baunez, C. and Robbins, T.W. (1999) Effects of dopamine depletion of the dorsal striatum and further interaction with subthalamic nucleus lesions in an attentional task in the rat. *Neuroscience*, 92(4): 1343–1356.
- Bonvento, G., Scatton, B., Claustre, Y. and Rouquier, L. (1992) Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. *Neurosci. Lett.*, 137(1): 101–104.
- Boulougouris, V., Dalley, J.W. and Robbins, T.W. (2007a) Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behav. Brain Res.*, 179(2): 219–228.
- Boulougouris, V., Glennon, J.C. and Robbins, T.W. (2007b) Dissociable effects of selective 5-HT(2A) and 5-HT(2C) receptor antagonists on serial spatial reversal learning in rats. *Neuropsychopharmacology*, In press, doi:10.1038/sj.npp.1301584.
- Brown, R.G. and Marsden, C.D. (1988) Subcortical dementia: the neuropsychological evidence. *Neuroscience*, 25(2): 363–387.
- Brown, V.J. and Bowman, E.M. (2002) Rodent models of prefrontal cortical function. *Trends Neurosci.*, 25(7): 340–343.
- Carli, M., Baviera, M., Invernizzi, R.W. and Balducci, C. (2006) Dissociable contribution of 5-HT1A and 5-HT2A receptors in the medial prefrontal cortex to different aspects of executive control such as impulsivity and compulsive perseveration in rats. *Neuropsychopharmacology*, 31(4): 757–767.
- Carli, M. and Samanin, R. (2000) The 5-HT(1A) receptor agonist 8-OH-DPAT reduces rats' accuracy of attentional performance and enhances impulsive responding in a five-choice serial reaction time task: role of presynaptic 5-HT(1A) receptors. *Psychopharmacology (Berl.)*, 149(3): 259–268.
- Castner, S.A., Williams, G.V. and Goldman-Rakic, P.S. (2000) Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. *Science*, 287(5460): 2020–2022.
- Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G. and Artigas, F. (2001) Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABA(A), and glutamate receptors. *J. Neurosci.*, 21(24): 9917–9929.
- Chamberlain, S.R., Muller, U., Blackwell, A.D., Clark, L., Robbins, T.W. and Sahakian, B.J. (2006a) Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science*, 311(5762): 861–863.
- Chamberlain, S.R., Muller, U., Deakin, J.B., Corlett, P.R., Dowson, J., Cardinal, R., Aitken, M.R., Robbins, T.W. and Sahakian, B.J. (2006b) Lack of deleterious effects of bupropion on cognition in healthy male volunteers. *J. Psychopharmacol.*, 21(2): 210–215.
- Chao, L.L. and Knight, R.T. (1995) Human prefrontal lesions increase distractibility to irrelevant sensory inputs. *Neuroreport*, 6(12): 1605–1610.
- Christakou, A., Robbins, T.W. and Everitt, B.J. (2001) Functional disconnection of a prefrontal cortical-dorsal striatal system disrupts choice reaction time performance: implications for attentional function. *Behav. Neurosci.*, 115(4): 812–825.
- Chudasama, Y. and Muir, J.L. (2001) Visual attention in the rat: a role for the prelimbic cortex and thalamic nuclei? *Behav. Neurosci.*, 115(2): 417–428.
- Chudasama, Y., Passetti, F., Rhodes, S.E., Lopian, D., Desai, A. and Robbins, T.W. (2003) Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behav. Brain Res.*, 146(1–2): 105–119.
- Chudasama, Y. and Robbins, T.W. (2003) Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *J. Neurosci.*, 23(25): 8771–8780.

- Chudasama, Y. and Robbins, T.W. (2004) Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: implications for schizophrenia. *Psychopharmacology (Berl.)*, 174(1): 86–98.
- Clarke, H.F., Dalley, J.W., Crofts, H.S., Robbins, T.W. and Roberts, A.C. (2004) Cognitive inflexibility after prefrontal serotonin depletion. *Science*, 304(5672): 878–880.
- Clarke, H.F., Walker, S.C., Crofts, H.S., Dalley, J.W., Robbins, T.W. and Roberts, A.C. (2005) Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J. Neurosci.*, 25(2): 532–538.
- Clarke, H.F., Walker, S.C., Dalley, J.W., Robbins, T.W. and Roberts, A.C. (2007) Cognitive inflexibility after prefrontal serotonin depletion is behaviorally and neurochemically specific. *Cereb. Cortex*, 17(1): 18–27.
- Cohen, J.D., Braver, T.S. and O'Reilly, R.C. (1999) A computational approach to prefrontal cortex, cognitive control and schizophrenia: recent developments and current challenge. In: Roberts A.C., Robbins T.W. and Weiskrantz L. (Eds.), *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, New York, pp. 195–220.
- Cole, B.J. and Robbins, T.W. (1987) Amphetamine impairs the discriminative performance of rats with dorsal noradrenergic bundle lesions on a 5-choice serial reaction time task: new evidence for central dopaminergic–noradrenergic interactions. *Psychopharmacology (Berl.)*, 91(4): 458–466.
- Cole, B.J. and Robbins, T.W. (1989) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: implications for theories of selective attention and arousal. *Behav. Brain Res.*, 33(2): 165–179.
- Collins, P., Roberts, A.C., Dias, R., Everitt, B.J. and Robbins, T.W. (1998) Perseveration and strategy in a novel spatial self-ordered sequencing task for nonhuman primates: effects of excitotoxic lesions and dopamine depletions of the prefrontal cortex. *J. Cogn. Neurosci.*, 10(3): 332–354.
- Consolo, S., Ramponi, S., Ladinsky, H. and Baldi, G. (1996) A critical role for D1 receptors in the 5-HT_{1A}-mediated facilitation of in vivo acetylcholine release in rat frontal cortex. *Brain Res.*, 707(2): 320–323.
- Cools, R., Barker, R.A., Sahakian, B.J. and Robbins, T.W. (2001) Enhanced or impaired cognitive function in Parkinson's disease as a function of dopaminergic medication and task demands. *Cereb. Cortex*, 11: 1136–1143.
- Cools, R., Clark, L., Owen, A.M. and Robbins, T.W. (2002) Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging. *J. Neurosci.*, 22(11): 4563–4567.
- Cools, R., Clark, L. and Robbins, T.W. (2004) Differential responses in human striatum and prefrontal cortex to changes in object and rule relevance. *J. Neurosci.*, 24(5): 1129–1135.
- Cools, R., Ivry, R.B. and D'Esposito, M. (2006) The human striatum is necessary for responding to changes in stimulus relevance. *J. Cogn. Neurosci.*, 18(12): 1973–1983.
- Crofts, H.S., Dalley, J.W., Collins, P., Van Denderen, J.C., Everitt, B.J., Robbins, T.W. and Roberts, A.C. (2001) Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. *Cereb. Cortex*, 11(11): 1015–1026.
- Dalley, J.W., Theobald, D.E., Eagle, D.M., Passetti, F. and Robbins, T.W. (2002a) Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. *Neuropsychopharmacology*, 26(6): 716–728.
- Dalley, J.W., Theobald, D.E., Pereira, E.A., Li, P.M. and Robbins, T.W. (2002b) Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. *Psychopharmacology (Berl.)*, 164(3): 329–340.
- Damasio, A.R. (1998) The somatic marker hypothesis and the possible functions of the prefrontal cortex. In: Roberts A.C., Robbins T.W. and Weiskrantz L. (Eds.), *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, New York, pp. 195–220.
- Day, J. and Fibiger, H.C. (1993) Dopaminergic regulation of cortical acetylcholine release: effects of dopamine receptor agonists. *Neuroscience*, 54(3): 643–648.
- Decary, A. and Richer, F. (1995) Response selection deficits in frontal excisions. *Neuropsychologia*, 33(10): 1243–1253.
- Del-Ben, C.M., Deakin, J.F., McKie, S., Delvai, N.A., Williams, S.R., Elliott, R., Dolan, M. and Anderson, I.M. (2005) The effect of citalopram pretreatment on neuronal responses to neuropsychological tasks in normal volunteers: an FMRI study. *Neuropsychopharmacology*, 30(9): 1724–1734.
- Di Matteo, V., De Blasi, A., Di Giulio, C. and Esposito, E. (2001) Role of 5-HT_{2C} receptors in the control of central dopamine function. *Trends Pharmacol. Sci.*, 22(5): 229–232.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Esposito, E. (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin_{2C} receptors. *Brain Res.*, 865(1): 85–90.
- Dias, R., Robbins, T.W. and Roberts, A.C. (1996) Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*, 380(6569): 69–72.
- Dias, R., Robbins, T.W. and Roberts, A.C. (1997) Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from “on-line” processing. *J. Neurosci.*, 17(23): 9285–9297.
- Drewe, E. (1975) Go-no go learning after frontal lobe lesions in humans. *Cortex*, 11(1): 8–16.
- Dubois, B., Boller, F., Pillon, B. and Agid, Y. (1991) Cognitive deficits in Parkinson's disease. In: Boller F. and Grafman J. (Eds.), *Handbook of Neuropsychology*, Vol. 5. Elsevier, Amsterdam.
- Eagle, D.M., Baunez, C., Hutcheson, D.M., Lehmann, O., Shah, A.P. and Robbins, T.W. (2007a) Stop-signal reaction time task performance: role of prefrontal cortex and subthalamic nucleus. *Cereb. Cortex*, 18(1): 178–185.
- Eagle, D.M. and Robbins, T.W. (2003) Lesions of the medial prefrontal cortex or nucleus accumbens core do not impair inhibitory control in rats performing a stop-signal reaction time task. *Behav. Brain Res.*, 146(1–2): 131–144.

- Eagle, D.M., Tufft, M.R., Goodchild, H.L. and Robbins, T.W. (2007b) Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology (Berl.)*, 192(2): 193–206.
- Ernst, M., Zametkin, A.J., Matochik, J.A., Jons, P.H. and Cohen, R.M. (1998) DOPA decarboxylase activity in attention deficit hyperactivity disorder adults. A [fluorine-18]fluorodopa positron emission tomographic study. *J. Neurosci.*, 18(15): 5901–5907.
- Evers, E.A., Cools, R., Clark, L., van der Veen, F.M., Jolles, J., Sahakian, B.J. and Robbins, T.W. (2005) Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. *Neuropsychopharmacology*, 30(6): 1138–1147.
- Fellows, L.K. and Farah, M.J. (2003) Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. *Brain*, 126: 1830–1837.
- Fillmore, M.T., Rush, C.R. and Hays, L. (2002) Acute effects of oral cocaine on inhibitory control of behavior in humans. *Drug Alcohol Depend.*, 67(2): 157–167.
- Fletcher, P.J., Grottick, A.J. and Higgins, G.A. (2002) Differential effects of the 5-HT_{2A} receptor antagonist M100,907 and the 5-HT_{2C} receptor antagonist SB 242,084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology*, 27(4): 576–586.
- Floresco, S.B., Magyar, O., Ghods-Sharifi, S., Vexelman, C. and Tse, M.T. (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology*, 31(2): 297–309.
- Florijn, W.J., Tarazi, F.I. and Creese, I. (1997) Dopamine receptor subtypes: differential regulation after 8 months treatment with antipsychotic drugs. *J. Pharmacol. Exp. Ther.*, 280(2): 561–569.
- Folstein, S.E., Brandt, J. and Folstein, M.F. (1990) Huntington's disease. In: Cummings J.L. (Ed.), *Subcortical Dementia*. Oxford University Press, New York.
- Frank, M.J., Santamaria, A., O'Reilly, R.C. and Willcutt, E. (2006) Testing computational models of dopamine and noradrenaline dysfunction in attention deficit/hyperactivity disorder. *Neuropsychopharmacology*, 32(7): 1583–1599.
- Freedman, M. (1990) Parkinson's disease. In: Cummings J.L. (Ed.), *Subcortical Dementia*. Oxford University Press, New York.
- Fuster, J. (1989) *The Prefrontal Cortex*. Raven Press, New York.
- Gauggel, S., Rieger, M. and Feghoff, T.A. (2004) Inhibition of ongoing responses in patients with Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry*, 75(4): 539–544.
- Gobert, A. and Millan, M.J. (1999) Serotonin (5-HT)_{2A} receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology*, 38(2): 315–317.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin_{2C} receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36(3): 205–221.
- Godefroy, O. and Rousseaux, M. (1996) Divided and focused attention in patients with lesion of the prefrontal cortex. *Brain Cogn.*, 30(2): 155–174.
- Goldman-Rakic, P.S. (1998) The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. In: Roberts A.C., Robbins T.W. and Weiskrantz L. (Eds.), *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, Oxford, UK, pp. 87–102.
- Granon, S., Passetti, F., Thomas, K.L., Dalley, J.W., Everitt, B.J. and Robbins, T.W. (2000) Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J. Neurosci.*, 20(3): 1208–1215.
- Haber, S.N., Fudge, J.L. and McFarland, N.R. (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *J. Neurosci.*, 20(6): 2369–2382.
- Hajós, M., Hajós-Korcsok, E. and Sharp, T. (1999) Role of the medial prefrontal cortex in 5-HT_{1A} receptor-induced inhibition of 5-HT neuronal activity in the rat. *Br. J. Pharmacol.*, 126(8): 1741–1750.
- Hampshire, A. and Owen, A.M. (2006) Fractionating attentional control using event-related fMRI. *Cereb. Cortex*, 16(12): 1679–1689.
- Harrison, A.A., Everitt, B.J. and Robbins, T.W. (1997) Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharmacology (Berl.)*, 133(4): 329–342.
- Harrison, A.A., Everitt, B.J. and Robbins, T.W. (1999) Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. *Behav. Brain Res.*, 100: 99–112.
- Hausknecht, K.A., San George, M., Gancarz, A.M., Ashrafioun, L., De Wit, H., Zhuang, Z. and Richards, J.B. (2006) Impulsivity in serotonin transporter knock-out mice: effects of methylphenidate. *Society for Neuroscience 2006*, Atlanta.
- Higgins, G.A., Enderlin, M., Haman, M. and Fletcher, P.J. (2003) The 5-HT_{2A} receptor antagonist M100,907 attenuates motor and “impulsive-like” behaviours produced by NMDA receptor antagonism. *Psychopharmacology (Berl.)*, 170: 309–319.
- Hollander, E. and Rosen, J. (2000) Impulsivity. *J. Psychopharmacol.*, 14: S39–S44.
- Hornak, J., O'Doherty, J., Bramham, J., Rolls, E.T., Morris, R.G., Bullock, P.R. and Polkey, C.E. (2004) Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *J. Cogn. Neurosci.*, 16(3): 463–478.
- Huber, S.J. and Shuttleworth, E.C. (1990) Neuropsychological assessment of subcortical dementia. In: Cummings J.L. (Ed.), *Subcortical Dementia*. Oxford University Press, New York.
- Idris, N.F., Repeto, P., Neill, J.C. and Large, C.H. (2005) Investigation of the effects of lamotrigine and clozapine in improving reversal-learning impairments induced by acute phencyclidine and D-amphetamine in the rat. *Psychopharmacology (Berl.)*, 179(2): 336–348.

- Inase, M., Li, B.M. and Tanji, J. (1997) Dopaminergic modulation of neuronal activity in the monkey putamen through D1 and D2 receptors during a delayed Go/Nogo task. *Exp. Brain Res.*, 117: 207–218.
- Iversen, S.D. and Mishkin, M. (1970) Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp. Brain Res.*, 11(4): 376–386.
- Jacobs, D.M., Levy, G. and Marder, K. (2003) Dementia in Parkinson's disease, Huntington's disease and related disorders. In: Feinberg T.E. and Farah M.J. (Eds.), *Behavioral Neurology and Neuropsychology* (2nd edn.). McGraw Hill, New York.
- Jin, J., Yamamoto, T. and Watanabe, S. (1997) The involvement of sigma receptors in the choice reaction performance deficits induced by phencyclidine. *Eur. J. Pharmacol.*, 319 (2–3): 147–152.
- Jones, B. and Mishkin, M. (1972) Limbic lesions and the problem of stimulus-reinforcement associations. *Exp. Neurol.*, 36(2): 362–377.
- Kirkby, D.L. and Higgins, G.A. (1998) Characterization of perforant path lesions in rodent models of memory and attention. *Eur. J. Neurosci.*, 10(3): 823–838.
- Koechlin, E., Ody, C. and Kouneiher, F. (2003) The architecture of cognitive control in the human prefrontal cortex. *Science*, 302(5648): 1181–1185.
- Koskinen, T., Ruotsalainen, S., Puumala, T., Lappalainen, R., Koivisto, E., Männistö, P.T. and Sirviö, J. (2000) Activation of 5-HT_{2A} receptors impairs response control of rats in a five-choice serial reaction time task. *Neuropharmacology*, 39(3): 471–481.
- Kruzich, P.J. and Grandy, D.K. (2004) Dopamine D2 receptors mediate two-odor discrimination and reversal learning in C57BL/6 mice. *BMC Neurosci.*, 5: p. 12.
- Kuroki, T., Meltzer, H.Y. and Ichikawa, J. (1999) Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *J. Pharmacol. Exp. Ther.*, 288(2): 774–781.
- Lange, K.W., Robbins, T.W., Marsden, C.D., James, M., Owen, A.M. and Paul, G.M. (1992) L-dopa withdrawal in Parkinson's disease selectively impairs cognitive performance in tests sensitive to frontal lobe dysfunction. *Psychopharmacology (Berl.)*, 107(2–3): 394–404.
- Lange, K.W., Sahakian, B.J., Quinn, N.P., Marsden, C.D. and Robbins, T.W. (1995) Comparison of executive and visuospatial memory function in Huntington's disease and dementia of Alzheimer type matched for degree of dementia. *J. Neurol. Neurosurg. Psychiatry*, 58(5): 598–606.
- Langley, K., Marshall, L., van den Bree, M., Thomas, H., Owen, M., O'Donovan, M. and Thapar, A. (2004) Association of the dopamine D4 receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. *Am. J. Psychiatry*, 161(1): 133–138.
- Lee, B., Groman, S., London, E.D. and Jentsch, J.D. (2007) Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys. *Neuropsychopharmacology*, 32(10): 2125–2134.
- Lewis, S.J., Slabosz, A., Robbins, T.W., Barker, R.A. and Owen, A.M. (2005) Dopaminergic basis for deficits in working memory but not attentional set-shifting in Parkinson's disease. *Neuropsychologia*, 43(6): 823–832.
- Lidow, M.S., Elsworth, J.D. and Goldman-Rakic, P.S. (1997) Down-regulation of the D1 and D5 dopamine receptors in the primate prefrontal cortex by chronic treatment with antipsychotic drugs. *J. Pharmacol. Exp. Ther.*, 281(1): 597–603.
- Lidow, M.S. and Goldman-Rakic, P.S. (1994) A common action of clozapine, haloperidol, and remoxipride on D1- and D2-dopaminergic receptors in the primate cerebral cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 91(10): 4353–4356.
- Lidow, M.S., Williams, G.V. and Goldman-Rakic, P.S. (1998) The cerebral cortex: a case for a common site of action of antipsychotics. *Trends Pharmacol. Sci.*, 19(4): 136–140.
- Linnoila, M., Virkkunen, M., Scheinin, M., Nuutila, A., Rimón, R. and Goodwin, F.K. (1983) Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life Sci.*, 33(26): 2609–2614.
- Logan, G.D. and Cowan, W.B. (1984) On the ability to inhibit thought and action: a theory of an act of control. *Psychol. Rev.*, 91(2): 295–327.
- MacLeod, C.M., Dodd, M.D., Sheard, E.D., Wilson, D.E. and Bibi, U. (2003) In opposition to inhibition. In: Ross B.H. (Ed.), *The Psychology of Learning and Motivation*. Academic Press, San Diego, CA, pp. 163–214.
- Masaki, D., Yokoyama, C., Kinoshita, S., Tsuchida, H., Nakatomi, Y., Yoshimoto, K. and Fukui, K. (2006) Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology (Berl.)*, 189(2): 249–258.
- Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H. and Weinberger, D.R. (2003) Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc. Natl. Acad. Sci. U.S.A.*, 100(10): 6186–6191.
- Mehta, M.A., Manes, F.F., Magnolfi, G., Sahakian, B.J. and Robbins, T.W. (2004) Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. *Psychopharmacology (Berl.)*, 176(3–4): 331–342.
- Mehta, M.A., Sahakian, B.J., McKenna, P.J. and Robbins, T.W. (1999) Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. *Psychopharmacology (Berl.)*, 146(2): 162–174.
- Mehta, M.A., Swainson, R., Ogilvie, A.D., Sahakian, J. and Robbins, T.W. (2001) Improved short-term spatial memory but impaired reversal learning following the dopamine D(2) agonist bromocriptine in human volunteers. *Psychopharmacology (Berl.)*, 159(1): 10–20.
- Meltzer, H.Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology*, 21: 106S–115S.
- Meltzer, H.Y., Matsubara, S. and Lee, J.C. (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin₂ pK_i values. *J. Pharmacol. Exp. Ther.*, 251(1): 238–246.
- Millan, M.J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J. Pharmacol. Exp. Ther.*, 295(3): 853–861.

- Millan, M.J., Dekeyne, A. and Gobert, A. (1998) Serotonin (5-HT)_{2C} receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. *Neuropharmacology*, 37(7): 953–955.
- Miller, E.K. and Cohen, J.D. (2001) An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.*, 24: 167–202.
- Moghaddam, B. and Bunney, B.S. (1990) Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. *J. Neurochem.*, 54(5): 1755–1760.
- Muir, J.L., Everitt, B.J. and Robbins, T.W. (1996) The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb. Cortex*, 6(3): 470–481.
- Nomura, M. and Nomura, Y. (2006) Psychological, neuroimaging, and biochemical studies on functional association between impulsive behavior and the 5-HT_{2A} receptor gene polymorphism in humans. *Ann. N.Y. Acad. Sci.*, 1086: 134–143.
- Nosarti, C., Rubia, K., Smith, A.B., Frearson, S., Williams, S.C., Rifkin, L. and Murray, R.M. (2006) Altered functional neuroanatomy of response inhibition in adolescent males who were born very preterm. *Dev. Med. Child Neurol.*, 48(4): 265–271.
- O'Doherty, J., Critchley, H., Deichmann, R. and Dolan, R.J. (2003) Dissociating valence of outcome from behavioral control in human orbital and ventral prefrontal cortices. *J. Neurosci.*, 23(21): 7931–7939.
- O'Doherty, J., Kringelbach, M.L., Rolls, E.T., Hornak, J. and Andrews, C. (2001) Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat. Neurosci.*, 4(1): 95–102.
- O'Neill, M. and Brown, V.J. (2007) The effect of striatal dopamine depletion and the adenosine A_{2A} antagonist KW-6002 on reversal learning in rats. *Neurobiol. Learn. Mem.*, 88(1): 75–81.
- Overtom, C.C., Verbaten, M.N., Kemner, C., Kenemans, J.L., van Engeland, H., Buitelaar, J.K., van der Molen, M.W., van der Gugten, J., Westenberg, H., Maes, R.A. and Koelega, H.S. (2003) Effects of methylphenidate, desipramine, and L-dopa on attention and inhibition in children with attention deficit hyperactivity disorder. *Behav. Brain Res.*, 145(1–2): 7–15.
- Owen, A.M., James, M., Leigh, P.N., Summers, B.A., Marsden, C.D., Quinn, N.P., Lange, K.W. and Robbins, T.W. (1992) Fronto-striatal cognitive deficits at different stages of Parkinson's disease. *Brain*, 115: 1727–1751.
- Owen, A.M., Roberts, A.C., Hodges, J.R., Summers, B.A., Polkey, C.E. and Robbins, T.W. (1993) Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. *Brain*, 116: 1159–1175.
- Owen, A.M., Roberts, A.C., Polkey, C.E., Sahakian, B.J. and Robbins, T.W. (1991) Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia*, 29(10): 993–1006.
- Parasuraman, R. (1998) The attentive brain: issues and concepts. In: Parasuraman R. (Ed.), *The Attentive Brain*. MIT Press, Cambridge, MA, pp. 3–15.
- Parasuraman, R. and Davies, D.R. (1977) A taxonomic analysis of vigilance. In: Mackie R.R. (Ed.), *Vigilance, Theory, Operational Performance and Physiological Correlates*. Plenum Press, New York.
- Park, S.B., Coull, J.T., McShane, R.H., Young, A.H., Sahakian, B.J., Robbins, T.W. and Cowen, P.J. (1994) Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. *Neuropharmacology*, 33(3–4): 575–588.
- Passetti, F., Chudasama, Y. and Robbins, T.W. (2002) The frontal cortex of the rat and visual attentional performance: dissociable functions of distinct medial prefrontal subregions. *Cereb. Cortex*, 12(12): 1254–1268.
- Passetti, F., Levita, L. and Robbins, T.W. (2003) Sulpiride alleviates the attentional impairments of rats with medial prefrontal cortex lesions. *Behav. Brain Res.*, 138(1): 59–69.
- Petrides, M. (1998) Specialized systems for the processing of mnemonic information within the primate frontal cortex. In: Roberts A.C., Robbins T.W. and Weiskrantz L. (Eds.), *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, Oxford, UK, pp. 103–114.
- Pincus, J.T. and Tucker, G. (2003) *Behavioral Neurology* (4th edn.). Oxford University Press, New York.
- Preuss, T.M. (1995) Do rats have a prefrontal cortex? The Rose-Woolsey-Akert Program reconsidered. *J. Cogn. Neurosci.*, 7: 1–24.
- Rahman, S., Sahakian, B.J., Hodges, J.R., Rogers, R.D. and Robbins, T.W. (1999) Specific cognitive deficits in mild frontal variant frontotemporal dementia. *Brain*, 122: 1469–1493.
- Ridley, R.M., Haystead, T.A. and Baker, H.F. (1981) An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol. *Pharmacol. Biochem. Behav.*, 14(3): 345–351.
- Rieger, M., Gauggel, S. and Burmeister, K. (2003) Inhibition of ongoing responses following frontal, nonfrontal, and basal ganglia lesions. *Neuropsychologia*, 17(2): 272–282.
- Robbins, T.W. (1998) Dissociable executive functions of the prefrontal cortex. In: Roberts A.C., Robbins T.W. and Weiskrantz L. (Eds.), *The Prefrontal Cortex: Executive and Cognitive Functions*. Oxford University Press, Oxford, UK, pp. 117–130.
- Robbins, T.W. (2000) Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp. Brain Res.*, 133(1): 130–138.
- Robbins, T.W. (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl.)*, 163(3–4): 362–380.
- Robbins, T.W. (2005) Chemistry of the mind: neurochemical modulation of prefrontal cortical function. *J. Comp. Neurol.*, 493(1): 140–146.

- Robbins, T.W. (2007) Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 362(1481): 917–932.
- Robbins, T.W. and Everitt, B.J. (1992) Functions of dopamine in the dorsal and ventral striatum. In: Robbins T.W. (Ed.), *Seminars in the Neurosciences*. Saunders, London, UK, pp. 119–127.
- Robbins, T.W., Muir, J.L., Killcross, A.S. and Pretsell, D. (1993) Methods for assessing attention and stimulus control in the rat. In: Sahgal A. (Ed.), *Behavioural Neuroscience: A Practical Approach*, Vol. 1. Oxford University Press, New York, pp. 13–47.
- Robbins, T.W. and Roberts, A.C. (2007) Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cereb. Cortex*, 17(Suppl. 1): 151–160.
- Roberts, A.C., De Salvia, M.A., Wilkinson, L.S., Collins, P., Muir, J.L., Everitt, B.J. and Robbins, T.W. (1994) 6-Hydroxydopamine lesions of the prefrontal cortex in monkeys enhance performance on an analog of the Wisconsin Card Sort Test: possible interactions with subcortical dopamine. *J. Neurosci.*, 14: 2531–2544.
- Rogers, R.D., Andrews, T.C., Grasby, P.M., Brooks, D.J. and Robbins, T.W. (2000) Contrasting cortical and subcortical activations produced by attentional-set shifting and reversal learning in humans. *J. Cogn. Neurosci.*, 12(1): 142–162.
- Rogers, R.D., Blackshaw, A.J., Middleton, H.C., Matthews, K., Hawtin, K., Crowley, C., Hopwood, A., Wallace, C., Deakin, J.F., Sahakian, B.J. and Robbins, T.W. (1999) Tryptophan depletion impairs stimulus-reward learning while methylphenidate disrupts attentional control in healthy young adults: implications for the monoaminergic basis of impulsive behaviour. *Psychopharmacology (Berl.)*, 146(4): 482–491.
- Rosvold, H.E., Mirsky, A.F., Sarason, I., Bransome, E.B. and Beck, L.H. (1956) A continuous performance test of brain damage. *J. Consult. Psychol.*, 20(5): 343–350.
- Rubia, K., Lee, F., Cleare, A.J., Tunstall, N., Fu, C.H., Brammer, M. and McGuire, P. (2005a) Tryptophan depletion reduces right inferior prefrontal activation during response inhibition in fast, event-related fMRI. *Psychopharmacology (Berl.)*, 179(4): 791–803.
- Rubia, K., Oosterlaan, J., Sergeant, J.A., Brandeis, D. and v Leeuwen, T. (1998) Inhibitory dysfunction in hyperactive boys. *Behav. Brain Res.*, 94(1): 25–32.
- Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S.C., Simmons, A., Andrew, C. and Bullmore, E.T. (2000) Functional frontalisation with age: mapping neurodevelopmental trajectories with fMRI. *Neurosci. Biobehav. Rev.*, 24(1): 13–19.
- Rubia, K., Russell, T., Overmeyer, S., Brammer, M.J., Bullmore, E.T., Sharma, T., Simmons, A., Williams, S.C., Giampietro, V., Andrew, C.M. and Taylor, E. (2001) Mapping motor inhibition: conjunctive brain activations across different versions of go/no-go and stop tasks. *Neuroimage*, 13(2): 250–261.
- Rubia, K. and Smith, A. (2004) The neural correlates of cognitive time management: a review. *Acta Neurobiol. Exp. (Warsaw)*, 64(3): 329–340.
- Rubia, K., Smith, A.B., Brammer, M.J., Toone, B. and Taylor, E. (2005b) Abnormal brain activation during inhibition and error detection in medication-naïve adolescents with ADHD. *Am. J. Psychiatry*, 162(6): 1067–1075.
- Schoenbaum, G., Nugent, S.L., Saddoris, M.P. and Setlow, B. (2002) Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations. *Neuroreport*, 13(6): 885–890.
- Schultz, W. and Dickinson, A. (2000) Neuronal coding of prediction errors. *Annu. Rev. Neurosci.*, 23: 473–500.
- Shallice, T. (1982) Specific impairments of planning. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 298(1089): 199–209.
- Solanto, M.V., Abikoff, H., Sonuga-Barke, E., Schachar, R., Logan, G.D., Wigal, T., Hechtman, L., Hinshaw, S. and Turkel, E. (2001) The ecological validity of delay aversion and response inhibition as measures of impulsivity in AD/HD: a supplement to the NIMH multimodal treatment study of AD/HD. *J. Abnorm. Child Psychol.*, 29(3): 215–228.
- Soubrié, P. (1986) Serotonergic neurons and behavior. *J. Pharmacol.*, 17(2): 107–112.
- Steele, T.D., Hodges, D.B., Jr., Levesque, T.R. and Locke, K.W. (1997) D1 agonist dihydroxydopamine releases acetylcholine and improves cognitive performance in rats. *Pharmacol. Biochem. Behav.*, 58(2): 477–483.
- Steinpreis, R.E. (1996) The behavioral and neurochemical effects of phencyclidine in humans and animals: some implications for modelling psychosis. *Behav. Brain Res.*, 74: 45–55.
- Sullivan, E.V., Lim, K.O., Mathalon, D., Marsh, L., Beal, D.M., Harris, D., Hoff, A.L., Faustman, W.O. and Pfefferbaum, A. (1998) A profile of cortical gray matter volume deficits characteristic of schizophrenia. *Cereb. Cortex*, 8(2): 117–124.
- Sutherland, N.S. and Mackintosh, N.J. (1971) *Mechanisms of Animal Discrimination Learning*. Academic Press, New York, NY.
- Talbot, P.S., Watson, D.R., Barrett, S.L. and Cooper, S.J. (2006) Rapid tryptophan depletion improves decision-making cognition in healthy humans without affecting reversal learning or set shifting. *Neuropsychopharmacology*, 31(7): 1519–1525.
- Tamminga, C.A., Thaker, G.K. and Medoff, D.R. (2002) Neuropsychiatric aspects of schizophrenia. In: Yudofski S.C. and Hales R.E. (Eds.), *Textbook of Neuropsychiatry and Clinical Neuroscience*. American Psychiatric Publishing, Washington, DC.
- Tunbridge, E.M., Bannerman, D.M., Sharp, T. and Harrison, P.J. (2004) Catechol-*o*-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J. Neurosci.*, 24(23): 5331–5335.
- Vaidya, C.J., Austin, G., Kirkorian, G., Ridlehuber, H.W., Desmond, J.E., Glover, G.H. and Gabrieli, J.D. (1998) Selective effects of methylphenidate in attention deficit hyperactivity disorder: a functional magnetic resonance study. *Proc. Natl. Acad. Sci. U.S.A.*, 95(24): 14494–14499.

- van den Wildenberg, W.P., van Boxtel, G.J., van der Molen, M.W., Bosch, D.A., Speelman, J.D. and Brunia, C.H. (2006) Stimulation of the subthalamic region facilitates the selection and inhibition of motor responses in Parkinson's disease. *J. Cogn. Neurosci.*, 18(4): 626–636.
- Vollm, B., Richardson, P., McKie, S., Elliott, R., Deakin, J.F. and Anderson, I.M. (2006) Serotonergic modulation of neuronal responses to behavioural inhibition and reinforcing stimuli: an fMRI study in healthy volunteers. *Eur. J. Neurosci.*, 23(2): 552–560.
- Wade, T.R., de Wit, H. and Richards, J.B. (2000) Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology (Berl.)*, 150(1): 90–101.
- Weinberger, D.R., Berman, K.F. and Daniel, D.G. (1991) Prefrontal cortex dysfunction in schizophrenia. In: Levin H.S., et al. *Frontal Lobe Function and Dysfunction*. Oxford University Press, New York.
- Weiner, I. and Feldon, J. (1986) Reversal and nonreversal shifts under amphetamine. *Psychopharmacology (Berl.)*, 89(3): 355–359.
- Winstanley, C.A., Chudasama, Y., Dalley, J.W., Theobald, D.E., Glennon, J.C. and Robbins, T.W. (2003) Intraprefrontal 8-OH-DPAT and M100907 improve visuospatial attention and decrease impulsivity on the five-choice serial reaction time task in rats. *Psychopharmacology (Berl.)*, 167(3): 304–314.
- Winstanley, C.A., Theobald, D.E., Dalley, J.W., Glennon, J.C. and Robbins, T.W. (2004) 5-HT_{2A} and 5-HT_{2C} receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-HT depletion. *Psychopharmacology (Berl.)*, 176(3–4): 376–385.

CHAPTER 26

Dopamine–serotonin interactions in attention-deficit hyperactivity disorder (ADHD)

Robert D. Oades*

Biopsychology Group, Clinic for Child and Adolescent Psychiatry and Psychotherapy, University of Duisburg-Essen, 45147 Essen, Germany

Abstract: Poor control of attention-related and motor processes, often associated with behavioural or cognitive impulsivity, are typical features of children and adults with attention-deficit hyperactivity disorder (ADHD). Until recently clinicians have observed little need to improve on or add to the catecholaminergic model for explaining the features of ADHD. Recent genetic and neuroimaging studies however provide evidence for separate contributions of altered dopamine (DA) and serotonin (5-HT) function in this disorder. Genetic studies imply that for both DA and 5-HT systems variants may frequently occur in ADHD for neurotransmitter uptake, synthesis and breakdown functions. The separate distributions in the brain of mesolimbic DA transporter and mesocortical DA D4 binding sites, both strongly implicated in ADHD, draws attention to potentially differential contributions from the 5-HT system. However, the evidence here points less towards an anatomical differentiation, as towards one in terms of inhibitory/facilitatory pre/post-synaptic location of receptors in the 5-HT₁ and 5-HT₂ families. While the monoamine metabolite levels excreted in ADHD are often correlated, this may well flow from a starting point where 5-HT activity is anomalously higher or lower than the generally lower than normal levels for DA. It appears that perhaps both situations may arise reflecting different diagnostic subgroups of ADHD, and where impulsive characteristics of the subjects reflect externalizing behaviour or cognitive impulsivity. For these features there is clear evidence that DA and 5-HT neuronal systems can and do interact anomalously in ADHD at the level of the soma, the terminals and at a distance. Interactions mediated by macroglia are also likely. However, it remains difficult to ascribe specific mechanisms to their effects (in potentially different subgroups of patients) from this relatively new field of study that has as yet produced rather heterogeneous results.

Keywords: attention; ADHD; dopamine; genetics; glia; impulsivity; prefrontal cortex; serotonin; venlafaxine

Introduction

The clinical problems of ADHD

The principle domains of dysfunction in this disorder are reflected in the name attention-deficit

hyperactivity disorder (ADHD) and may be found in nearly 10% of children worldwide (Faraone et al., 2003). It is widely agreed that the constituent characteristics represent extremes of features normally distributed across the population. Indeed, the high heritability of the disorder at ca. 70% (Faraone et al., 2005) provides a basis for the genetic strategy of investigating risk factors, known as the quantitative trait locus approach

*Corresponding author. Tel.: +49-(0)201-9597-030;
Fax: +49-(0)201-7227-302; E-mail: robert.oades@uni-due.de

(Asherson, 2004). This has the potential to link the categorical disorder to continuously distributed traits associated closely with the underlying genetic liability in the general population.

There is a subtype of ADHD where the domains of overactivity, restlessness and behavioural impulsivity predominate (hyperactive-impulsive or ADHDhi), and another, an inattentive subtype (ADHDin), where poor executive attention and cognitive impulsivity predominate. But for most of the cases seeking professional help these features are found together in the combined type (ADHDct). These features are not expressed all the time. The DSM IV manual (American Psychiatric Association) describes them as ‘often present’: in laboratory studies one notes a high intra-individual variability in the measures taken (Scheres et al., 2001; Russell et al., 2006). In all clear diagnoses a clinical impairment is noted.

Some cases are markedly withdrawn showing low self-esteem, others show frequent outbursts of affect, many are characterized by both of these ‘internalizing’ and ‘externalizing’ traits and most have problems in social and academic environments. Frequently these problems are diagnosed as comorbid (e.g. oppositional and conduct disorder). Onset is usually in mid- or early childhood and affects boys more than girls (ca. 3-5 to 1, Buitelaar et al., 2006). In about a third of cases the disorder persists into adulthood and the gender ratio evens out (Biederman et al., 2004).

Dopamine not serotonin dysfunction?

Consensus suggests that in one form or another dopamine (DA) activity is lower than normal in children and adolescents with ADHD (Levy, 2004; Iversen and Iversen, 2007). Thus, based on the knowledge that intimate interactions between DA and serotonin (5-HT) occur widely in the mammalian brain (see previous chapters) one would intuitively expect — as cause or effect — that there would be some changes in the activity of 5-HT in cases with ADHD. One should first ask why this idea has to date had little resonance with the psychologists and psychiatrists who study ADHD.

Key evidence for the view that central 5-HT activity is irrelevant to explanations of ADHD derives from the success of the medication usually prescribed. Long- or short-acting forms of methylphenidate improve the problems in 60–70% of both younger and older ADHD patients (Wigal et al., 2004; Biederman et al., 2007a). Merely the domain of the problem (e.g. restless motor activity, poor social interactions and attention-related cognition) is differentially sensitive to dose (Pelham and Murphy, 1990). The overall proportion of patients improving with treatment rises to around 80% if another psychostimulant such as amphetamine is considered (Committee on children and disabilities and committee on drugs, 1996). Methylphenidate inhibits the reuptake of DA and noradrenalin (NA), but has no *direct* effect on 5-HT (Leonard et al., 2004). The present discussion does not consider further the role of NA activity that undoubtedly also contributes to cerebrocortical dysfunction in ADHD (Oades, 2005). Successful medication is apparently not acting on 5-HT systems and the clinician is happy with such a good response rate to these agents. Certainly the dogma, promulgated in older reviews (Oades, 1987; Zametkin and Rapoport, 1987), has long been that one does not need to consider 5-HT to explain clinical observations, or the results of laboratory examinations of ADHD behaviour.

However, the argument for the catecholamine and against the 5-HT contribution to ADHD is somewhat superficial. It would seem important to seek an explanation for why around 30% of patients are non-responders, and seek reasons for why a large proportion of ‘responders’ show far less than 50% improvement. Most children with ADHD show little or no improvement of academic performance or social function (Abikoff et al., 2004; Gualtieri and Johnson, 2008). Indeed the striking improvement seen after methylphenidate treatment in the NIMH multimodal treatment study over the first year of the study dwindled to the very modest levels recorded after intensive psychotherapy over 2–3 years (Jensen and Arnold, 2004). In seeking an explanation it is appropriate to suggest that 5-HT or a quite

different component of CNS function may be playing a significant role.

Evidence for an altered dopaminergic and serotonergic contribution to ADHD

Dopamine (DA)

First, it is useful to recall briefly that the activities of DA and 5-HT are associated with the expression of ADHD when considered separately. Investigations to provide direct evidence of neurotransmitter involvement in ADHD have usually not considered the role of more than one transmitter. These studies and indirect evidence for interactions are discussed in Putative dopamine and serotonin interactions in ADHD (below). Examples of key evidence focusing on DA (here) and 5-HT (see section Serotonin (5-HT)) are selected from the fields of neuroimaging and genetics.

Volkow et al. (2007b) describe a comparison of D2/D3 receptor availability in medication-naïve adults with ADHD with healthy subjects using positron emission tomography (PET) and the D2 ligand [¹¹C]raclopride. They recorded a low availability of binding sites in the left caudate nucleus on placebo. After methylphenidate treatment there was a blunted bilateral response in the striatum that extended to the limbic region of the amygdala and hippocampus. Similar PET/SPECT studies report a decrease of the DA transporter in the basal ganglia and thalamus (Hesse et al., 2006; Volkow et al., 2007a). Volkow relates how recent replications have resolved some of the differences with earlier conflicting reports. Of great interest is the extension of the findings of ‘hypodopaminergia’ from striatal to both limbic and thalamic regions (Fig. 1). In addition to the much-studied striatum, the thalamus is a major component of the fronto-striatal circuits where DA-modulated dysfunction is often invoked as a basis for cognitive problems in ADHD (Swanson et al., 2007) and where the DA transporter (DAT) is normally abundant (Telang et al., 1999; Garcia-Cabezas et al., 2007, Fig. 2). It may be noted that the usually extrasynaptic locus of DAT

is here well suited to control the influence of DA on the moderate-to-dense 5-HT innervation of the thalamus from the raphe nuclei (Morrison and Foote, 1986; Vertes, 1991).

The above reports are of particular interest as the authors related the neurochemical PET changes registered with some of the clinical features of their patients. For example, the blunted limbic (raclopride-binding) response to medication and the dopamine transporter (DAT1) binding in the putamen were significantly related to ratings of inattention on the Conners scale (Volkow et al., 2007a, b; Fig. 1).

The current state of genetic studies does not offer much support for unusual polymorphisms affecting D2/3 receptor function (but see Nyman et al., 2007), yet does imply that variants of the DA D4 receptor, abundant in mesocortical regions, and the DA transporter (DAT1), abundant in mesolimbic/striatal regions, are associated with features of ADHD. The former (D4) seems to be important for the ‘inattentive’ and the latter (DAT1) for the ‘hyperactive-impulsive’ part of the clinical spectrum of ADHD (Diamond, 2007). Note: DAT1 removes most of the unused extracellular DA in subcortical regions, whereas in the cortices >60% of the DA is removed by the catabolic enzyme catechol-*o*-methyltransferase, COMT.

Central to numerous studies of the DAT1 has been the association with ADHD of the 10-repeat allele of a variable number tandem repeat (VNTR) in the 3'-untranslated region of the DA transporter gene. Some recent meta-analyses have played down the strength of this association. However, the reason lies with a confound usually overlooked in most reports, namely that the 10-repeat allele concerned in fact tags a nearby functional variant, the 6-repeat allele of another VNTR in intron 8. This has been replicated in the IMAGE cohort that now includes a total of 1159 children with ADHD (Asherson et al., 2007).

Serotonin (5-HT)

Few have seriously investigated the putative involvement of 5-HT in ADHD. Thus, there are few studies with neuroimaging techniques to

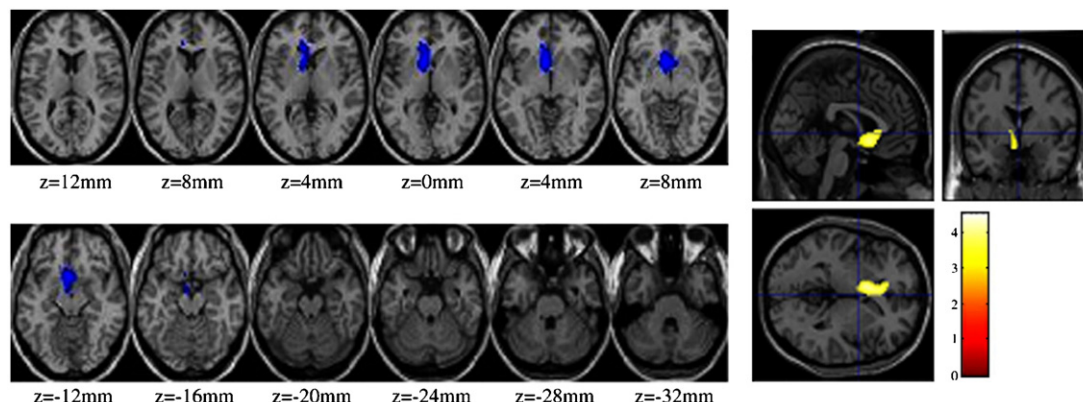
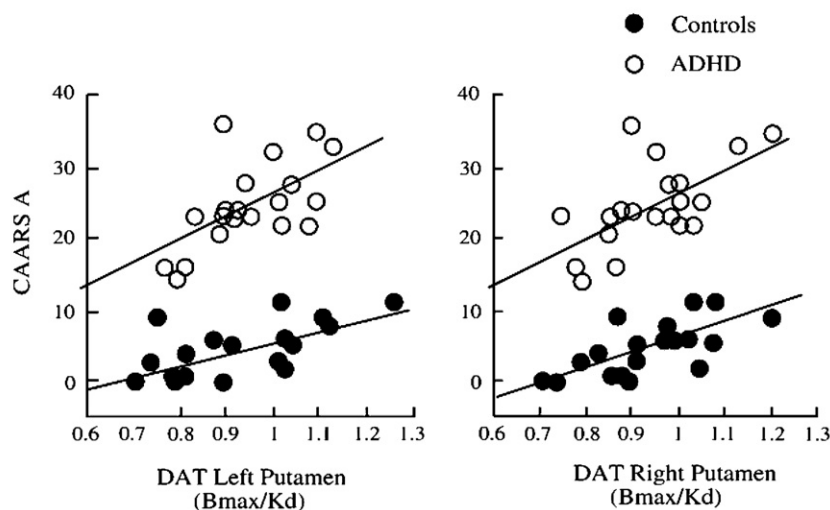
A/**B/**

Fig. 1. (A) Regions centred on the nucleus accumbens and hypothalamus where levels of DAT were significantly higher in healthy controls than adults with ADHD. (B) Regression slopes between DAT availability (right and left putamen) and ratings of inattention (Conners scales) in adults with ADHD. They show severer symptoms at any given level of DAT in the patients. Adapted with permission from Volkow et al. (2007a) and Elsevier Ltd. (See Color Plate 26.1 in color plate section.)

compare the potential contribution of 5-HT activity with that described for DA in the previous section. Hesse et al. (2006) report no evidence for unusual binding characteristics of the 5-HT transporter (SERT) in their SPECT study (^{123}I -FP-CIT) of the midbrain and brainstem of adult ADHD patients. However, Riiikonen et al. (2005) used the radioligand ^{123}I -labelled nor-CIT, which specifically labels SERT with a 10-fold higher affinity than the DA transporter, in their

study of children with ADHD and foetal alcohol syndrome. They reported significantly less binding (25%) in the anterior cingulate cortex, but found no reductions in the temporal cortices or in the midbrain. More studies are required to avoid the confounds of co-morbidity, preferably with the use of high-affinity ligands that are necessary to provide clear results (Elfving et al., 2007).

Other approaches indeed suggest that some aspects of SERT activity do not function well in

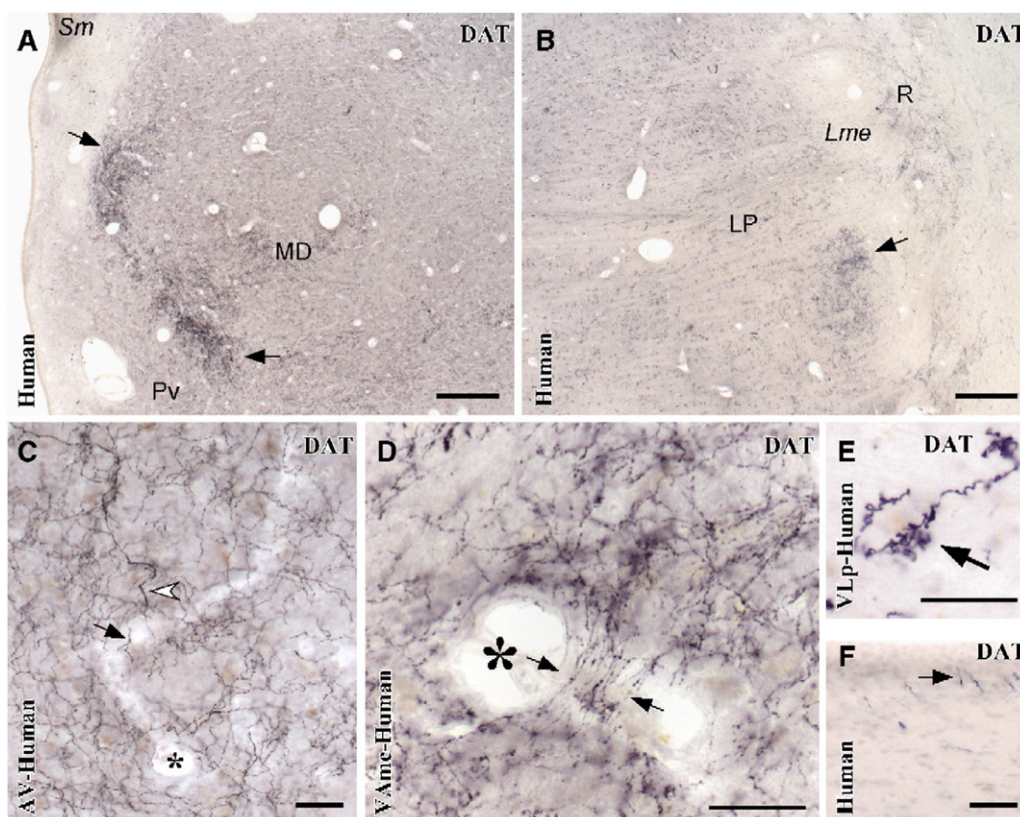


Fig. 2. Dense and uneven immunolabelling of DAT in the human thalamus is shown, and lies in proximity to the innervation from the raphe nuclei (see text). DAT labelling A/B in associative medio-dorsal (MD) and lateral posterior (LP) nuclei (calibration 400 μ M), C/D in limbic antero-ventral (AV) and motor ventro-anterior (VAmc) and posterior ventro-lateral (VLp) nuclei (calibration 40 μ M). Adapted with permission from Garcia-Cabezas et al. (2007) and Elsevier Ltd.

ADHD. For example, Oades et al. (2002) describe an increased affinity (reduced K_d) for platelet SERT-binding measured with paroxetine in children with ADHD. The platelet model appears to mimic the situation in the CNS (Cheetham et al., 1993). This was associated with the characteristically poor attention and performance shown on the stop-signal task that was also correlated with ratings of distractibility and impulsivity. This is illustrated in Fig. 3, which also shows the opposite relationship observed for ratings of (impulsive) outbursts of aggression. Much earlier reduced binding of 3 H-imipramine to NA and 5-HT uptake sites was reported for pre-pubertal children with ADHD and conduct disorder (Stoff et al., 1987). Further, several genetic studies claim that short or long forms of the transport promoter region that

show reduced/enhanced transcriptional efficiency are preferentially transmitted in ADHD (Biederman and Faraone, 2005; Curran et al., 2005; Li et al., 2007). However, some recent negative findings (Wigg et al., 2006; Guimaraes et al., 2007) clearly underline the need to differentiate between subgroups in future investigations. A recent brief review of the heterogeneous genetics literature on other features of the 5-HT system (Oades, 2007) concluded that there was tentative support for association of alleles with the 5-HT_{1B} receptor in cases of the predominantly inattentive subtype, and with the 5-HT_{2A/C} receptor(s) in subjects showing more hyperactivity and impulsivity. This review also describes evidence for inefficient 5-HT synthesis in ADHD that relates to transmission of a variant for the enzyme tryptophan hydroxylase (TPH2).

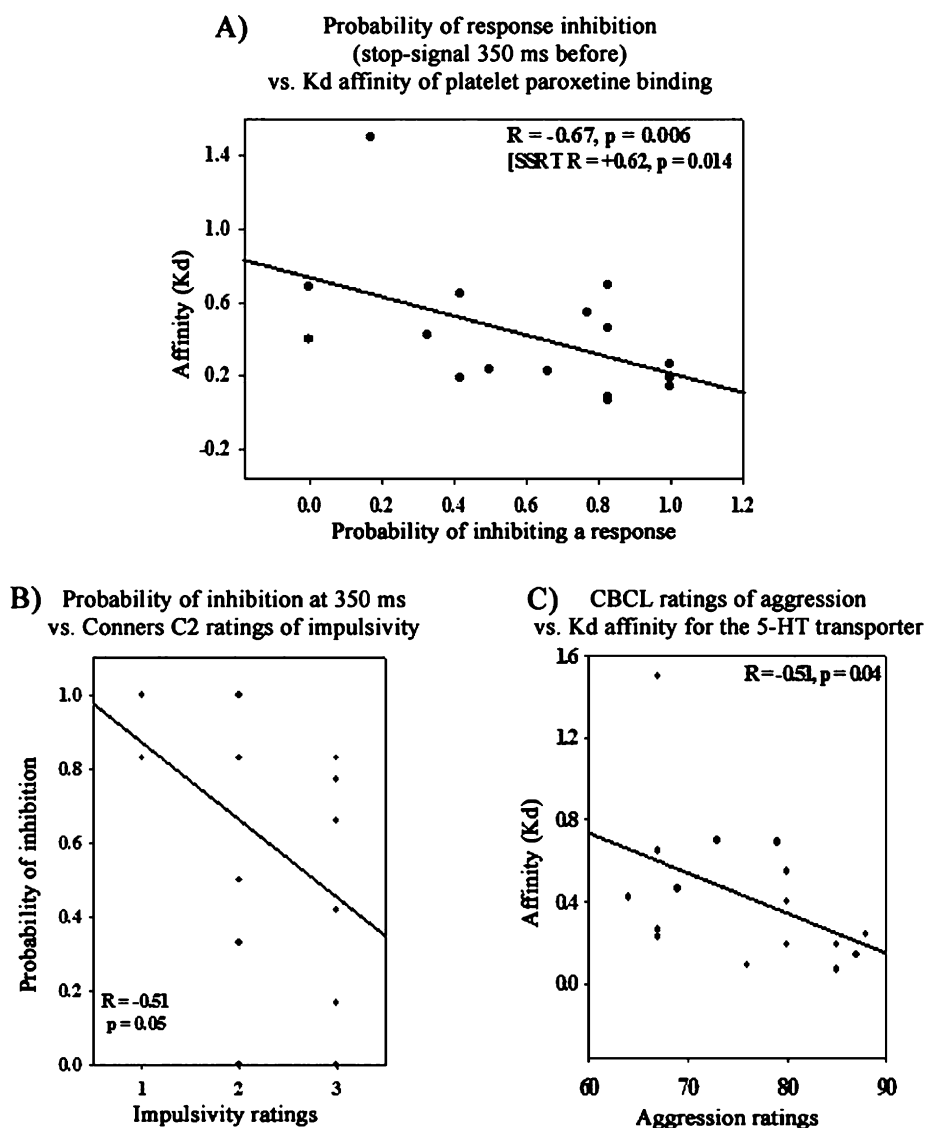


Fig. 3. In children with ADHD, (A) the affinity (K_d) for 5-HT uptake in platelets vs. the probability of response inhibition on the stop-task (stop-signal 350 ms prior to the mean response time); (B) the probability of stop-task inhibition vs. the ratings of impulsivity on the Conners scale and (C) the affinity of 5-HT uptake vs. child behaviour checklist (CBCL) rating of aggressive behaviour. Adapted with permission from Oades et al. (2002) and Taylor and Francis Ltd.

Putative dopamine and serotonin interactions in ADHD

Introduction

The overlap of ascending 5-HT and DA projections to both subcortical and cortical territories,

like the putative roles of 5 types of DA receptor and perhaps 22 types of the 5-HT receptor, is discussed in detail elsewhere in this volume (Di Matteo et al., 2001; Martin-Ruiz et al., 2001; Meneses, 1999; Michelsen et al., 2008). However, as yet, the only functional outcome one can discuss in the context of ADHD is that the numerous and

varying types of interaction appear to have an effect, and that the nature of this effect can be described merely on a rather large scale with poor resolution. As a contribution of 5-HT to ADHD has largely been ignored, there is a near absence of studies designed to examine its potential specific interactions with DA. Thus, this review largely focuses on those studies that have at least considered both transmitters: where a change of activity reflecting both monoamines has been recorded, an interaction is inferred.

Nonetheless such putative interactions are based on solid anatomical evidence for the frequent convergence of these two monoaminergic systems (Phelix and Broderick, 1995). It is perhaps useful in the following discussion to hold three categories or types of interaction in mind. (1) At the level of the neuron or pathway, changes of DA activity can affect 5-HT responsivity, with inhibition in terminal regions (Consolo et al., 1996; Di Matteo et al., 2001) or disinhibition at the autoreceptor level (Mendlin et al., 1999). (2) Stimulation or blockade of 5-HT neurons can influence DA responsivity, especially through blockade or stimulation of psychostimulant-induced release of mesolimbic DA (Porras et al., 2002; Di Giovanni et al., 2008). (3) Alternatively, a functional interaction may occur at a distance, mediated by an intermediate neuron.

Some specific examples of these sorts of interaction are as follows. (1) Damage to DA systems in neonatal rats leads to a large increase of 5-HT release in the basal ganglia (Luthman et al., 1989). (2) Blockade of 5-HT₂ binding sites leads to increased DA outflow as measured by PET records of raclopride-binding in baboons (Dewey et al., 1995). These examples illustrate the traditional view of the mutual inhibition of the activity of these two monoamines. As models they are both pertinent to potential explanations of the situation in ADHD (see impulsive cognition and aggression in the Serotonin (5-HT) section above). This disguises the nature of other forms of interaction that occur in detail and vary with the pre- vs. post-synaptic location of different types of receptor in cell body (midbrain) or mesolimbic/cortical projection regions (Millan et al., 2007).

An important and significant example of influence ‘at a distance’ (the third type of 5HT-DA

interaction) is the facilitation of ascending DA transmission via stimulation of 5-HT_{1b} receptors on GABA interneurons in the midbrain (Millan et al., 2007). However, there are numerous other potentially significant ‘influences at a distance’, as illustrated by the striofugal pathways to parts of the pallidum (Levesque and Parent, 2005). Not only can a major 5-HT input modulate the influence of DA activity directly in the organization of behavioural modules in the striatum, but it can also have a ‘second go’ through the heavy innervation to the pallidum at the start of the final common pathway (Napier and Istre, 2007). Analogously, the motor cortices also receive a major input from 5-HT projections whose activity facilitates gross motor output (Jacobs and Fornal, 1995). Surprisingly, none of these features of CNS circuitry have received much attention in the context of the restlessness, hyperactivity and clumsiness often associated clinically with ADHD or in laboratory studies of impaired fine motor control (Meyer and Sagvolden, 2006; Rommelse et al., 2007). An indication that there is a DA/5-HT interaction affecting motor control is that the ratio of their metabolites measured in CSF has been reported to correlate positively with ratings of motor activity (Castellanos et al., 1994). However, in this particular case inferences should be qualified by noting that the relationship reported was strongly driven by the DA metabolite.

The concept of influence at a distance has widespread implications when one considers the 5-HT innervation extending from the midbrain raphe nuclei to a series of subcortical regions (e.g. habenula) or cortical territories (e.g. cingulate) that then feedback directly to the origin of DA pathways in the ventral tegmental area (Oades and Halliday, 1987). This principle also operates for regions innervated by DA.

Studies on both dopamine and serotonin in investigations of ADHD

Genetics

Meta-analysis of genetic studies of ADHD has shown support, from at least three reports each (Faraone et al., 2005), for an association between

the disorder and variants of several DA and 5-HT receptors (D4, D5, DAT1, 5-HT_{1B} and SERT). Arguably, more important and relevant to the present consideration of DA/5-HT interactions are findings from within one large cohort (IMAGE, Brookes et al., 2006). This is because reports of associations with DA and 5-HT variants within one cohort provide a good starting point for eventually demonstrating their joint importance in individuals. The IMAGE team found adjusted gene-wide significance for variants affecting DA uptake (DAT1), 5-HT synthesis (TPH2) and monoamine breakdown (MAO-A) based on 674 families with 776 child and adolescent cases of ADHDct. Nominal significance extended to D4, 5-HT_{1E} and dopa decarboxylase (DDC) that is involved in both DA and 5-HT synthesis. In this study of 51 candidate genes involved in monoaminergic transmission, fatty acid synthesis and circadian rhythms, nominal significance extended to 12 other genes less relevant to this discussion, but importantly not to the remaining 33 genes. Considering that a study of the heterogeneity in the IMAGE population demonstrated the appropriateness of the North European contribution to this cohort (Neale et al., 2007), there is a reasonably firm basis for claiming that aspects of both 5-HT and DA activity contribute to the variance in ADHD. Indeed our current family-based association analyses of impulsivity in the IMAGE cohort suggest associations of variants for 5-HT_{1E} and TPH2 genes with both cognitive and behavioural impulsivity; also SERT, 5-HT_{2A}, D1 and the DA transporter DAT1 were associated with the former and DA D4 with the latter type of impulsiveness (Oades et al., in submission). However, only now are various laboratories tackling the nature of the dependence of an individual phenotype on interactions between 5-HT and DA.

Broadly supportive of part of the IMAGE study is the recent report from Ribases et al. (2007) on adult and childhood cases of ADHD. They claim an association with ADHD for certain single-nucleotide polymorphisms (SNP) in the genes controlling expression of DDC (chromosome 7), the 5-HT_{2A} receptor (chromosome 13) and the X-linked monoamine oxidase (MAO-B). Corrected significant results for DDC and 5-HT_{2A} were

based on 451 child and adult cases and for MAO on 188 adult cases. This is intriguing as DDC and MAO-B are involved in the synthesis and breakdown respectively of *both* DA and 5-HT. Indeed there is some marginally significant support elsewhere for the DDC result with variants reported from a similar location (Hawi et al., 2001). Neuroimaging studies also offer some support for anomalies in monoamine synthesis involving DDC. PET measures of fluorodopa, where inter-regional ratios index DDC activity, were reported to be reduced in both adult and childhood ADHD cases, albeit in different regions. Nonetheless these indices of DDC activity related to diagnostically relevant features, namely, the behavioural and hyperactive symptoms (Ernst et al., 1998, 1999). Using a simple additive model, Ribases et al. estimated that the combined effect of their three risk haplotypes contributed 5.2% of the adult ADHD phenotypic variance and the DDC and 5-HT_{2A} genetic variants accounted for 2.3% of child ADHD variability.

Few studies have looked for an association of MAO-B with ADHD, and these have found no association (Domschke et al., 2005). However, further research is required on this region on the X chromosome, as there is an extensive overlap for sequences determining MAO-A and MAO-B. A number of polymorphisms for MAO-A and its promoter region have been examined, and there are several reports of preferential transmission of longer, active and shorter, less active forms, depending on the putative aetiology of the types of patient studied (Oades, 2007).

In the current context, the finding of the over-representation of a specific 5-HT_{2A} haplotype (G-C-C) in adult and childhood cases of the ADHDct subtype (Ribases et al., 2007), is of special interest as this receptor type is frequently located on DA neurons. While at least five other studies have not been able to find associations with ADHD for several variants affecting this receptor, there are a large number of SNPs available for study. An association with impulsivity on the Barratt rating scale in alcohol-dependent patients has been described (Preuss et al., 2001). Indeed, Reuter et al. (2006) report that one allele (T102C) was significantly associated with ratings of

hyperactivity and impulsivity in a group of healthy adults (Nomura et al., 2006). This is intriguing as in the same cohort there were strong correlations for the met/met allele of the enzyme COMT (active in mesocortical DA breakdown) with hyperactive and impulsive as well as inattentive subgroups. This implies potentially a separate, as well as a joint, influence of the two monoamines on the phenotype. These findings are of further interest due to the following reasons: (1) such ratings could apply to ADHDct and ADHDhi subtypes; (2) the association with ADHDhi provides an interesting counterpart to associations of the 5-HT_{1B} receptor with the ADHDin subgroup (Hawi et al., 2002; Smoller et al., 2006; Oades, 2007); (3) the results remind one of the blockade by 5-HT_{2A} antagonists of hyperlocomotion induced by DA stimulation in animals (O'Neill et al., 1999; Porras et al., 2002; Bishop et al., 2005). The 5-HT_{1B} and 5-HT_{2A} receptors are contrasted here as animal studies suggest that, respectively, they are frequently pre-synaptic and inhibitory, and post-synaptic and excitatory in location and function (Millan et al., 2007).

A number of genetic studies have been directed to SERT, and in particular, have concentrated on long and short forms in the promoter region. This is of nominal interest to this analysis of DA/5-HT interactions, as SERT is also capable of transporting DA. Indeed, genetically speaking all three monoamine transporters share a 50% sequence homology (Gainetdinov and Caron, 2003). Some (but not all) studies of SERT transmission have found associations of short (Li et al., 2007) and long variants in the promoter region (Kent et al., 2002) or the 12-repeat allele in the intron 2 VNTR (Banerjee et al., 2006) with ADHD expression. Oades (2007) suggested that the heterogeneity of results reported for these markers may reflect the need for defining more closely the subtype(s) and comorbidities expressed in the patients examined. For example, there is a well-established relationship for externalizing behaviour and conduct disorders often comorbid with ADHD with low 5-HT activity (Flory et al., 2007; van Goozen et al., 2007), platelet 5-HT uptake mechanisms (Stadler et al., 2004) and long/short forms of the SERT promoter region (Cadoret et al., 2003). Variations of some of these

measures also seem to reflect social stratification and geographic origin (Manuck et al., 2005; Li et al., 2007). However, only the study from Schmidt et al. (2007) has directly concerned the interaction with DA mechanisms. In their review of the literature they found that the presence of one to two copies of the short allele of the SERT gene and the long allele (7-repeat allele) version of the DA D4 gene predicted internalizing- and externalizing-related behaviours, respectively. In their own work they reported that normal 7-year-old children with this genetic combination did indeed show more externalizing and internalizing behaviour than children with any other combination of long and short alleles. In contrast, those children with the long SERT form, as well as the long DA D4 genotypes, had the lowest reported scores on internalizing and externalizing behaviours. Such an interaction suggests that the long SERT form could, in such circumstances, have a protective function.

Evidence from genetic studies implicates the neuroprotective and neurodisruptive roles of 5-HT in ADHD. This could involve inhibitory and excitatory profiles under the control of receptors of the 5-HT₁ and 5-HT₂ families. However, as the results as yet remain heterogeneous, one cannot rule out that both may be implicated through a common aetiological problem with monoamine synthesis (cf. DDC and TPH2 results above). It is therefore natural that the present discussion should now proceed on to what is known about the activity of the neurotransmitter 5-HT itself.

Neurochemistry

It would be helpful for improving an understanding of the neurobiology of potential DA/5-HT interactions in ADHD to have measures of monoamine metabolism, or at least the results of pharmacological challenges on the release of hormones known to be controlled by these monoamines. These have naturally, for ethical reasons, been carried out rarely with minors, and the study of adults with ADHD remains in its infancy.

Nonetheless, a few studies are relevant for the interest in the 5-HT_{1B} and 5-HT_{2A} sites implicated

above from genetic work. A group of children showing both oppositional defiance disorder as well as ADHD were given a challenge dose of sumatriptan that is an agonist at several 5-HT₁ binding sites, especially the 5-HT_{1B} receptor (Snoek et al., 2002). Compared to control children, the patients proved to be twice as sensitive to the challenge dose in terms of the growth hormone response. While this result implicates the 5-HT_{1B} site, it also suggests that the effect may have been achieved through excitatory post-synaptic sites rather than the more usual inhibitory pre-synaptic sites. In the second report Schulz et al. (1998) compared the cortisol and prolactin response to fenfluramine in ADHD children with and without fathers with alcohol problems. While both groups showed similar increases of prolactin, only the former risk group showed a marked increase in cortisol. The authors point out that as both 5-HT₂ and 5-HT₁ sites influence cortisol release, but only the 5-HT₂ sites affect prolactin, the conclusion must be that the 5-HT_{2A/C} sites were not responding anomalously. Nonetheless considering that NA and 5-HT uptake blockade through desipramine can be clinically helpful (Gastfriend et al., 1985; Donnelly et al., 1986), and can also speed responses on a stop task and increase prolactin levels in ADHD children (Overtoom et al., 2003), taken together the results do suggest that the 5-HT₂ site may influence processes normally approached by pharmacotherapy with methylphenidate alone.

Together, these studies support a potential involvement of 5-HT activity in ADHD, arguably by way of 5-HT₁ if not also via the 5-HT₂ receptor family, at least in a subgroup of patients. However, it is important to ask whether unusual levels of 5-HT availability and metabolism at these receptors interact with DA (or vice versa) in ADHD. On the whole, levels of the DA and 5-HT metabolites (homovanillic acid, HVA and 5-hydroxyindoleacetic acid, 5-HIAA) measured in the CSF of patients are intercorrelated. If symptoms are severe then HVA levels are often high. If levels are high, they predict a good response to psychostimulant treatment and subsequently fall: falling HVA levels are followed by those for 5-HIAA (Castellanos et al., 1996). HVA levels are

not always high in ADHD, but low levels predict a poor treatment response. Low levels of 5-HIAA in the CSF are associated with episodes of aggression in children, adolescents (Kruesi et al., 1990) and non-human primates (Higley et al., 1996). Unexpectedly, these studies could report no relationship for HVA with aggression. By contrast, in aggressive rats anticipating an encounter, microdialysis of mesolimbic regions demonstrated rising DA and falling 5-HT release (Ferrari et al., 2003). However, of course, the rodent and primate situations are not exactly comparable.

It is interesting that the Castellanos CSF studies reported that there was no correlation between the monoamine metabolites they measured and indices of cognition and accuracy on a continuous performance task (CPT). However, they did find that the more controversial peripheral levels of the metabolites measured in urine correlated with those in the CSF. This lends support to the claim that urinary indices of monoamine metabolism are reflecting both somatic and brain sources. Here, in urinary sources the HVA/5-HIAA ratio was reported as being significantly lower in ADHD children than in healthy controls (Oades and Müller, 1997). The skew seemed to be driven by the higher levels of the 5-HT metabolite. Uzbekov (2006) confirmed not only that HVA excretion was relatively low in children with ADHD, but also that response to treatment with sydnocarb was accompanied by markedly reduced levels of 5-HIAA.

In summary it would seem that there is at least a subgroup of cases of ADHD where 5-HT systems are more active than normal and this can be partially corrected by stimulating DA activity. The discussion now proceeds to consider whether these markers of the apparent involvement of 5-HT receptors and their activity in ADHD are associated with the activity of the brain and neuropsychological function where the DA innervation plays a role.

Neuropsychology (neuroimaging)

A recent review catalogued an increasing degree of influence of central 5-HT activity in ADHD across the field of attention-related processes from the

treatment of salient stimuli (exogenous attention) over the inter-regional selective processes (endogenous attention) to cognitive impulsivity that reflects poor executive attentional control (Oades, 2007). It is therefore reasonable to examine first whether these processes reflect cognitive mechanisms that methylphenidate also influences through promoting catecholaminergic activity.

Certainly, on various versions of the CPT that reflect sustained attentional processes methylphenidate speeds responses, and the effect is blocked by antipsychotic agents (Levy, 1991; Levy and Hobbes, 1996). A contribution of DA is implicated. Slow latencies and more impulsive errors of commission in ADHD have been associated with the short allele of the DA D4 receptor (Manor et al., 2002), a 148 bp allele of the D5 gene (Manor et al., 2004), the 9- and 10-repeat alleles of the DA transporter (Loo et al., 2003) and the highly active valine allele of COMT (Eisenberg et al., 2003). In turn these have been associated with improvements following methylphenidate treatment (Manor et al., 2002, 2004). Indeed CPT indices of inattention and impulsivity have been linked to PET measures of DA receptor sensitivity and availability when challenged with methylphenidate (Rosa-Neto et al., 2005). [However, it is instructive to note that 'improvement' does not mean that task performance normalized (Tucha et al., 2005).] These reports complement the aforementioned contribution from 5-HT, where high activity (5-HIAA) impairs, and decreases relate to improved signal detection measures and performance (Oades, 2002; Overtom et al., 2003). Poor task performance can be associated with a variant of the TPH2 gene influencing 5-HT performance (−703 G/T; Reuter et al., 2007). The potential for an interaction between these monoaminergic systems is supported by improvements after methylphenidate treatment in those ADHD cases that carried risk variants of TPH2 for 5-HT synthesis and showed poor CPT performance in terms of speed of processing, reaction time variability and errors of omission (Manor et al., 2008).

A recent report from Rubia et al. (2007) concentrated on a set of tasks in which ADHD subjects have often been reported to make errors of commission. They found that cognitive impulsivity

and an increased variability of response was the dominant overall result from administering a battery of six tasks where each tested a different aspect of inhibitory control in young people with ADHD. Not only do such tasks habitually engage frontal regions in the right hemisphere, but Rubia et al. (2004) also showed that decreased activity in these regions in healthy subjects who had taken a tryptophan-depleting drink was associated with trials on which they had difficulty to withhold a response as required. Several laboratories have found that after taking such a drink that restricts 5-HT synthesis, normal people do experience a range of difficulties in making stimulus–response associations, acquiring a reversal learning task and indeed show an impulsive style (Park et al., 1994; Walderhaug et al., 2002).

Delay avoidance or the preference for immediate over delayed reward, even if it is larger, is a typical feature of childhood behaviour and is exaggerated in many of those with ADHD: it has been described as the consequence of the failure of an *impulsive* child to engage effectively with delay-rich environments (Sonuga-Barke, 2005). Arguably, the phenomenon is related to the steeper reinforcement gradients attributed to ADHD children (Sagvolden et al., 2005). That is, a stimulus and the appropriate response have to occur closer together in time for an association to be acquired both in the animal model and for children with ADHD. There is wide agreement on the basis of animal studies that the choice of, and switching to, an alternative stimulus for response depends on its salience, the perceived adaptiveness of a new situation or, very often, the anticipated reward: this involves bursts of DA activity (Oades, 1985; Goto et al., 2007; Roesch et al., 2007). It is less widely appreciated that to elicit such shifts requires region-specific 5-HT participation (Winstanley et al., 2006; van der Plasse and Feenstra, 2007). The role here is likely that of modulating the gain of the signal (Oades, 2006). While these authors emphasize mechanisms taking place in mesocortical projection regions (medial and orbito-frontal cortices) (Floden et al., 2008) it may be noted that treatments that also influence subcortical regions will be involved in these DA/5-HT interactions. Thus, if the DA

transporter is knocked out in rodents, reinforcement as measured by cocaine administration (Mateo et al., 2004) or by conditioned place preference to amphetamine (Budygin et al., 2004) remains, until a 5-HT_{1A} antagonist is administered. Indeed, stimulation of 5-HT_{2C} receptors actually attenuates the cocaine-induced release of DA from the rat's nucleus accumbens (Navailles et al., 2008) while 5-HT_{1B} stimulation, as an example of gain modulation, enhances place preference responses for cocaine (Barot et al., 2007). Clearly both DA and 5-HT systems were involved from the outset in mechanisms largely mediated by the mesolimbic system. Further, illustrating that the interaction can work both ways, Banks et al. (Banks et al., 2008) reported that SERT availability is markedly increased in monkeys experienced in cocaine self-administration. These results could pertain to the vulnerability of ADHD patients for succumbing to substance misuse, where a bidirectional risk for co-morbidity is in fact apparent (Biederman et al., 2007b).

However, underlying the reward aspects of risky decision making, there is an important component of information processing contributing to an 'impulsive response style'. Errors of commission made during a CPT task are regarded conventionally as a classic indicator of cognitive impulsivity. If children with ADHD persist in making such errors then one should consider whether there is anything amiss with the processing of their responses, and the feedback designating the response as an error. Some authors report that the increase in response latency normally seen in the first correct response after an error is often missing in those with ADHD (Schachar et al., 2004), until treated with methylphenidate (Krusch et al., 1996). It is then natural to ask about the neurophysiological response in this situation. As yet, it is still too early to resolve the conflicting reports on the nature of the neurophysiological ERP responses recorded after the errors made by ADHD children (i.e. error-related negativity and positivity). Studied on different types of task, the negative response has been reported to be larger (Burgio-Murphy et al., 2007), normal (Wiersema et al., 2005) or reduced (Van Meel et al., 2007). However, a participation of both DA and 5-HT projections in the neurophysiological response to

an error of commission is evident in apparently healthy subjects. Here, the presence of one or two copies of the low-activity, short SERT promoter allele is associated with larger negative and positive ERPs following an error (Fallgatter et al., 2005). The early negative ERP response also becomes larger in subjects treated with amphetamine (De Bruijn et al., 2004).

This section has indicated that while DA and 5-HT interact in some of the endogenous mechanisms involved in selecting information for further processing, the best defined of these incur executive attentional control, where poor function results in cognitive impulsivity and variability.

Studies of medication show signs of interactions in ADHD

Given that the efficacy of catecholamine reuptake blockade in ADHD is well established (see section Evidence for an altered dopaminergic and serotonergic contribution to ADHD), it is appropriate to consider evidence about medication that affects the 5-HT system. There is considerable anecdotal experience suggesting that venlafaxine, an inhibitor at SERT and to a lesser degree the NA transporter (Gould et al., 2006), can present an effective treatment, especially with adult patients with ADHD (Hedges et al., 1995; Findling et al., 1996; Popper, 2000). Open trials report a response rate of 50–78%, which is comparable with psychostimulants (Maidment, 2003; Findling et al., 2007). The primary reason for its use is reflected in its anti-depressant profile. But as noted above, while lability of mood and affect is often a feature of ADHD, so also is the variability of behaviour in a wider context. Indeed, the control by 5-HT activity of impulsive responses, whether of a cognitive or aggressive nature, represents a potential target for pharmacotherapy, albeit reflecting a need for alterations in different directions. Past experience with desipramine (inhibitor of NA and 5-HT uptake; Maidment, 2003) and tranylcypromine (two enantiomers involved in inhibition of MAO), and interference with monoamine uptake (Baker et al., 1991), is also relevant even though their prescription is now restricted due to the adverse side-effects.

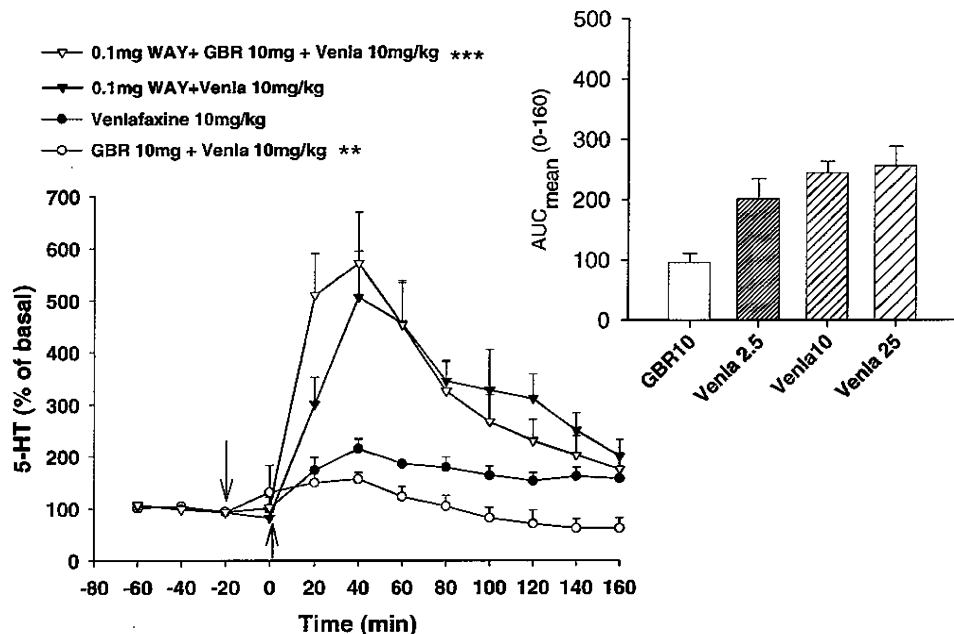
Evidence pointing to the relevance of 5-HT/DA interactions comes from pharmacological and neurobiological studies. Weikop et al. (2007a, b) reported initially surprising results from microdialysis experiments using the frontal cortex of rats following combinations of treatments with agents that block reuptake. Adjunctive treatment of the specific 5-HT reuptake inhibitor citalopram with methylphenidate resulted in a much larger increase of DA over that recorded following methylphenidate treatment alone, but a marked reduction of 5-HT release compared to treatment with citalopram alone. These effects were not evident in the nigrostriatal system. The authors suggested that the effect on DA could reflect a local elevation of 5-HT tone resulting in disinhibition in the ventral tegmental area. Normally, such an autoreceptor (or pre-synaptic) effect might be expected to stimulate 5-HT_{1A} receptors that would increase DA release, and increase burst firing in the mesolimbic and mesocortical projections (Millan et al., 2007). With systemic administration of other 5-HT uptake inhibitors, post-synaptic effects of 5-HT₁ stimulation can be expected that would result in decreased DA neuronal activity (Di Mascio et al., 1998). Weikop et al. (2007a) also reported on the effect of blocking DA reuptake (with GBR 12909) at the same time as treating their animals with systemic venlafaxine. GBR 12909 alone had no effect on mesocortical monoamine levels. However, venlafaxine alone can increase DA, NA and 5-HT levels by 136–256%, reminiscent of the effect of tranylcypromine, while inhibiting firing in the dorsal raphe and locus ceruleus (Haddjeri et al., 2004). The combination (as above) raised DA levels and reduced 5-HT levels further (Fig. 4). As noted above, the underlying mechanism could reflect post-synaptic activation of receptors in the 5-HT_{1/3/7} families, blockade of the 5-HT_{2A} site and/or long-loop feedback to GABA neurons in the brainstem nuclei (Weikop et al., 2007a). Direct evidence is still required. Whichever way the studies are viewed, there is the strong implication that venlafaxine can influence DA/5-HT interactions in a way that can result in improvements in ADHD. There remain many questions of how this happens in detail.

It is noteworthy that a PET study of the effect of venlafaxine on brain glucose metabolism, which naturally focused on depressed patients, described marked decreases of metabolic activity in the orbito-frontal and medial-frontal regions (Kennedy et al., 2007). This is of interest, firstly because these regions overlap with those found in animal studies of DA and 5-HT activity changes in impulsive behaviour on delayed reinforcement tasks (Winstanley et al., 2006; see section on Neuropsychology (Neuroimaging)). Secondly, also on the topic of impulsive responses, the effect of tryptophan depletion in young healthy adults (Rubia et al., 2004, see above) not only reduced activity in these frontal regions, but also increased right occipito-temporal activity.

Lastly, also on the subject of energy metabolism, it seems appropriate to mention one hypothetical locus for DA/5-HT interactions in ADHD that has hardly received any attention. Russell et al. (2006) proposed a re-direction of research effort to achieve a better understanding of the energy supply via the lactate shuttle from glia to neurons. They suggested that the variability of behavioural responsiveness in ADHD, previously mentioned in association with impulsivity, could be explained by a lack of energy from astrocyte sources to sustain rapid or burst firing in neurons when required. They also extended the hypothesis to account for delayed maturation and myelination in the CNS of those with ADHD (Shaw et al., 2007) and attributed this to a lack of energy and precursor supply from the oligodendrocytes. To understand the relevance here it is important to realize that most DA receptors have been localized on these glial cells (D1, D3, D4, D5; Miyazaki et al., 2004). Increased levels of catecholamines, facilitated by methylphenidate treatment, stimulate glycolysis (Todd and Botteron, 2001). So what is the function of the 5-HT receptors also identified on astrocytes, namely 5-HT_{1A}, B, D, F, 5-HT_{2A}, B, C, 5-HT₆ and 5-HT₇ (Hirst et al., 1998; Doherty and Pickel, 2001)?

The question of the nature of the function of monoaminergic binding sites on glia has hardly been tackled. However, it is not surprising that initial results suggest that the activation of 5-HT_{1A} sites in the amygdala and prefrontal regions can tone down the release of lactate stimulated by an environmental

(a)



(b)

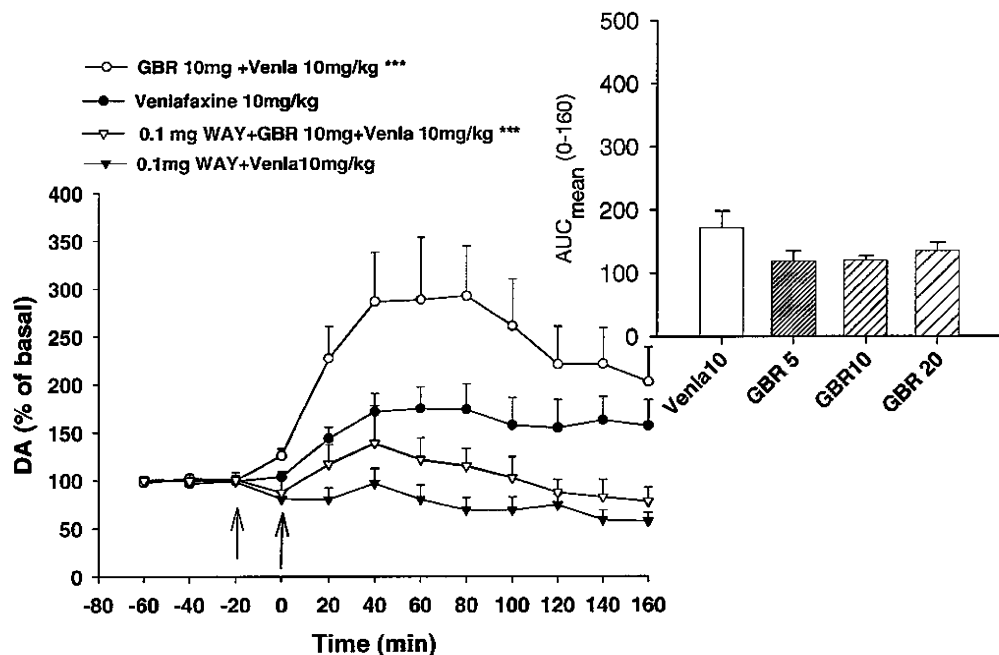


Fig. 4. (a) Frontal 5-HT levels after venlafaxine alone (10 mg/kg, ip at 0 min) or with GBR12909 (10 mg/kg, sc at -20 min), or WAY-100635 (0.1 mg/kg, sc at -20 min) or all three substances. The inset shows the effects of three doses of venlafaxine co-administered with GBR12909 with respect to the area under the curve (0-160 min) for controls. (b) Frontal DA levels after the same four treatment as (a). The inset shows the effects of three doses of GBR12909 co-administered with venlafaxine. Data are expressed as the area under the curve (0-160 min) values of the control group, mean SEM, neonatal rats, *** $P < 0.001$; ANOVA, Fisher's PLSD-test: ** $P < 0.02$ (vs. venlafaxine alone). Adapted with permission from Weikop et al. (2007a) and Sage Publications Ltd.

stressor (Uehara et al., 2006). This was demonstrated by blockade of the effects of tandospirone with a specific 5-HT_{1A} antagonist. However, as the antagonist did not interfere with the similar effects of perospirone, it is possible that the affinity of this drug for 5-HT_{2A} and D2 sites may have come into play and also modulated the supply of energy. Also relevant in the context of this section are the effects reported to follow treatment of an astroglia–microglia culture with venlafaxine (Vollmar et al., 2007). They found that after provoking an inflammatory situation, venlafaxine promoted an augmentation of anti-inflammatory cytokines transforming growth factor-beta (transforming growth factor-beta, TGF- β) and reduced levels of the pro-inflammatory cytokines (interleukin-6, IL-6 and gamma-interferon, IFN- γ). Thus, it would seem likely that venlafaxine exerted anti-inflammatory effects that could have been due to the increased levels of monoamines that the treatment induced. The potential significance for ADHD is that a predominance of the pro-inflammatory cytokines would otherwise bias the metabolism of tryptophan towards neurotoxic metabolites such as quinolinic acid (Myint et al., 2007).

In this section the idea has been put forward that combining pharmacological treatments that influence both the DA and 5-HT systems may have differential even opposite effects on the release and availability of these two monoamines, and that this can be associated with beneficial consequence for ADHD pathology. However, there is still a need for controlled studies of this claim. A further challenge requiring detailed study is to find out whether the purported consequences of DA and 5-HT uptake blockade result primarily from neuronal neurophysiology and/or glial energetics.

Discussion and conclusions

For a consideration of 5-HT/DA interactions and their putative dysfunction in ADHD there are three major CNS territories of interest: the mesostriatal, the mesolimbic and the mesocortical. In the mesostriatal (and mesothalamic) domain there are two features of special neurobiological interest relating to the nature of DA/5-HT

interactions. Compared to the other DA projection systems, this is where the distribution of the DA transporter predominates. This is also where the 5-HT innervation primarily derives from the dorsal raphe. The anatomical nature of this input differs from that deriving from the median raphe in that it is construed to be better at volume control than at advancing specific synaptic control of the target regions (Vertes, 1991; Michelsen et al., 2007).

The mesostriatal/thalamic mode of action contrasts with the situation in mesocortical projection regions. Here, extracellular DA availability is more under the control of synaptic COMT, and the release of 5-HT, mostly of median raphe origin is more localized with the aid of clusters of boutons around the target neurons (Michelsen et al., 2007). The characteristics of mesolimbic structures lie between these two extremes, with the innervation of specific parts of the hippocampus or amygdala arising predominantly from one or the other raphe complex (Michelsen et al., 2008). The generalizations proposed here must be tempered by an awareness of a considerable overlap of these two modes of innervation. For example, far more 5-HT of dorsal raphe origin is released in the frontal than in posterior cortices: there is an inverse trend for 5-HT with origin in the median raphe.

One of the more salient difficulties in focusing on the contribution of 5-HT/DA interactions in ADHD is the evident contribution of components of 5-HT controlled processes to the expression of frequently comorbid conditions such as conduct disorder (and its associated externalizing, aggressive characteristics). Short variants of the SERT promoter are associated with lower levels of SERT expression and high levels of extrasynaptic 5-HT. These features have been reported to have some association with signs of aggression and conduct disorder in young males (Cadoret et al., 2003; Beitchman et al., 2006) but also, infrequently, with ADHD (Cadoret et al., 2003; Li et al., 2007). In view of the unequivocal association of ADHD and features of the DA system, one might speculate that a search for genetic associations between aspects of both monoamines and young people diagnosed with ADHD vs. conduct disorder would help disentangle the relative contributions of these two monoamines. Perhaps the tagging of function to the

COMT gene is an example. For example, COMT-deficient mice, if male, are aggressive, rather as in humans (Gogos et al., 1998): this forms a parallel to the association of the *met* allele in Chinese ADHD patients, if male (Qian et al., 2003).

This review describes evidence for a role for the 5-HT₁ and 5-HT₂ families of receptors in the interaction between the 5-HT and DA projection systems, and in some of the dysfunctions evident in ADHD. Descriptions of clearly too much or too little activity are often difficult to elucidate where it remains uncertain whether post- or pre-synaptic activity predominates, or an inhibitory interneuron permits disinhibition in the control of specific functions. Each is possible in the context of the expression of ADHD in an individual, on the one hand, as comorbid with conduct disorder or in another individual as being of the inattentive type. This has been illustrated by the contrast of behavioural vs. cognitive impulsivity. More detailed neurobiological studies are necessary. Nonetheless, an understanding of the basic anatomical features (above) does provide a basis for prediction and further detailed investigation. For example, immunocytochemical work shows that more 5-HT_{1A} labelled dendrites in the ventral tegmental area are found in the nucleus parabrachialis than in the nucleus paranigralis (Doherty and Pickel, 2001). This suggests that 5-HT_{1A} stimulation is more likely to influence mesocortical than subcortical DA function. Indeed, stimulation of these sites can have anomalous influences on DA function and executive attentional processes, such as those impaired in ADHD.

Until recently, clinicians have seen little need to improve on the catecholaminergic model for explaining the features of ADHD. Recent genetic and neuroimaging studies, however, provide evidence for separate contributions of altered DA and 5-HT function in this disorder. Genetic studies imply that for both DA and 5-HT systems variants may frequently occur in ADHD for neurotransmitter uptake (DAT1, SERT), synthesis (TPH2, DDC) and breakdown functions (MAO and perhaps COMT). The mesolimbic (striatal) distribution of DAT1 and the mesocortical abundance of D4 binding sites, both strongly implicated in

ADHD, draw attention to the possibility of differential contributions from the 5-HT system. Here the evidence points not so much to region-specific anomalies as a differentiation in terms of inhibitory/facilitatory pre-/post-synaptic location of receptors in the 5-HT₁ and 5-HT₂ families. Whether these receptor-based changes are secondary to the processes controlling transmitter availability is a question that remains to be answered. While levels of activity and metabolism (HVA and 5-HIAA) are often correlated, this may well flow from a starting point where 5-HT activity is anomalously higher or lower than the generally lower than normal levels for DA. It appears that perhaps both situations may arise reflecting different subgroups of ADHD, and where impulsive characteristics reflect externalizing behaviour or cognitive impulsivity. This differentiation on a dimensional level, however, has yet to be studied systematically on the nosological level.

Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
ADHD	attention-deficit hyperactivity disorder
COMT	catecholamine- <i>o</i> -methyl transferase
CPT	continuous performance task
DA	dopamine
DAT1	dopamine transporter
DDC	dopa decarboxylase
HVA	homovanillic acid
IFN- γ	gamma-interferon
IL-6	interleukin-6
NA	noradrenalin
SERT	serotonin transporter
SNP	single-nucleotide polymorphisms
TGF- β	transforming growth factor-beta
TPH	tryptophan hydroxylase
VNTR	variable number tandem repeat

References

- Abikoff, H.B., Hechtman, L., Klein, R.G., Gallagher, R., Fleiss, K., Etcovitch, J., Cousins, L., Greenfield, B., Maertn,

- D. and Pollack, S. (2004) Social functioning in children with ADHD treated with long-term methylphenidate and multimodal psychosocial treatment. *J. Am. Acad. Child. Adolesc. Psychiatry*, 43: 820–829.
- Asherson, P. (2004) Attention deficit hyperactivity disorder in the post-genomic era. *Eur. Child. Adolesc. Psychiatry*, 13: 50–66.
- Asherson, P., Brookes, K.-J., Franke, B., Chen, W., Gill, M., Ebstein, R.P., Buitelaar, J., Banaschewski, T., Sonuga-Barke, E.J.S., Eisenberg, J., Manor, I., Miranda, A., Oades, R.D., Roeyers, H., Rothenberger, A., Sergeant, J.A., Steinhausen, H.-C. and Faraone, S.V. (2007) Confirmation that a specific haplotype of the dopamine transporter gene is associated with combined type ADHD. *Am. J. Psychiatry*, 164: 674–677.
- Baker, G.B., Bornstein, R.A., Rouget, A.C., Ashton, S.E., Van Muiyden, J.C. and Coutts, R.T. (1991) Phenylethylaminergic mechanisms in attention-deficit disorder. *Biol. Psychiatry*, 29: 15–22.
- Banerjee, E., Sinha, S., Chatterjee, A., Gangopadhyay, P.K., Singh, M. and Nandogopal, K. (2006) A family-based study of Indian subjects from Kolkata reveals allelic association of the serotonin transporter intron-2 (STin2) polymorphism and attention-deficit-hyperactivity disorder (ADHD). *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 141: 361–366.
- Banks, M.L., Czoty, P.W., Gage, H.D., Bounds, M.C., Garg, P.K., Garg, S. and Nader, M.A. (2008) Effects of cocaine and MDMA self-administration on serotonin transporter availability in monkeys. *Neuropsychopharmacology*, 33: 219–225.
- Barot, S.K., Ferguson, S.M. and Neumaier, J.F. (2007) 5-HT_{1B} receptors in nucleus accumbens efferents enhance both rewarding and aversive effects of cocaine. *Eur. J. Neurosci.*, 25: 3125–3131.
- Beitchman, J.H., Baldassarra, L., Mik, H., De Luca, V., King, N., Bender, D., Ehteshami, S. and Kennedy, J.L. (2006) Serotonin transporter polymorphisms and persistent, pervasive childhood aggression. *Am. J. Psychiatry*, 163: 1103–1105.
- Biederman, J. and Faraone, S.V. (2005) Attention-deficit hyperactivity disorder. *Lancet*, 366: 237–248.
- Biederman, J., Faraone, S.V., Monuteaux, M., Bober, M. and Cadogan, E. (2004) Gender effects on attention-deficit/hyperactivity disorder in adults, revisited. *Biol. Psychiatry*, 55: 692–700.
- Biederman, J., Mick, E.O., Surman, C., Doyle, R., Hammerness, P., Michel, E., Martin, J. and Spencer, T.J. (2007a) Comparative acute efficacy and tolerability of OROS and immediate release formulations of methylphenidate in the treatment of adults with attention-deficit/hyperactivity disorder. *BMC Psychiatry*, 7: p. 49.
- Biederman, J., Petty, C.R., Wilens, T.E., Faraone, S.V., Purcell, C.A., Mick, E., Monuteaux, M.C. and Faraone, S.V. (2007b) Familial risk analyses of attention deficit hyperactivity disorder and substance use disorders. *Am. J. Psychiatry*, 165: 107–115.
- Bishop, C., Daut, G.S. and Walker, P.D. (2005) Serotonin 5-HT_{2A} but not 5-HT_{2C} receptor antagonism reduces hyperlocomotor activity induced in dopamine-depleted rats by striatal administration of the D₁ agonist SKF 82958. *Neuropharmacology*, 49: 350–358.
- Brookes, K.-J., Xu, X., Chen, W., Zhou, K., Neale, B.M., Lowe, N., Aneley, R., Franke, B., Gill, M., Ebstein, R.P., Buitelaar, J., Sham, P., Cambell, D., Knight, J., Andreou, P., Altink, M., Arnold, R., Boer, F., Buschgens, C., Butler, L., Christiansen, H., Feldman, L., Fleischman, K., Fliers, E., Howe-Forbes, R., Goldfarb, A., Heise, A., Gabriels, I., Lubetzki, I., Marco, R., Medad, S., Minderaa, R.B., Mulas, F., Müller, U.C., Mulligan, A., Rabin, K., Rommelse, N.N.J., Sethna, V., Sorohan, J., Uebel, H., Psychogiou, L., Weeks, A., Barrett, R., Craig, I., Banaschewski, T., Sonuga-Barke, E.J.S., Eisenberg, J., Kuntsi, J., Manor, I., McGuffin, P., Miranda, A., Oades, R.D., Plomin, R., Roeyers, H., Rothenberger, A., Sergeant, J.A., Steinhausen, H.-C., Taylor, E.A., Thompson, M.J., Faraone, S.V., Asherson, P. and Johansson, L. (2006) Analysis of 51 candidate genes in DSM-IV combined subtype attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. *Mol. Psychiatry*, 11: 934–953.
- Budygin, E.A., Brodie, M.S., Sotnikova, T.D., Mateo, Y., John, C.E., Cyr, M., Gainetdinov, R.R. and Jones, S.R. (2004) Dissociation of rewarding and dopamine transporter-mediated properties of amphetamine. *Proc. Natl. Acad. Sci. U.S.A.*, 101: 7781–7786.
- Buitelaar, J.K., Barton, J., Danckaerts, M., Friedrichs, E., Gillberg, C., Hazell, P.L., Hellems, H., Johnson, M., Kalverdijk, L.J., Masi, G., Michelson, D., Revol, O., San Sebastian, J., Zhang, S. and Zuddas, A. (2006) A comparison of North American versus non-North American ADHD study populations. *Eur. Child. Adolesc. Psychiatry*, 15: 177–181.
- Burgio-Murphy, A., Klorman, R., Shaywitz, S.E., Fletcher, J.M., Marchione, K.E., Holahan, J., Stuebing, K.K., Thatcher, J.E. and Shaywitz, B.A. (2007) Error-related event-related potentials in children with attention-deficit hyperactivity disorder, oppositional defiant disorder, reading disorder, and math disorder. *Biol. Psychol.*, 75: 75–86.
- Cadoret, R.J., Langbehn, D., Caspers, K., Troughton, E.P., Yucuis, R., Sandhu, H.K. and Philibert, R. (2003) Associations of the serotonin transporter promoter polymorphism with aggressivity, attention deficit, and conduct disorder in an adoptee population. *Compr. Psychiatry*, 44: 88–101.
- Castellanos, F.X., Elia, J., Kruesi, M.J.P., Gulotta, C.S., Mefford, I.N., Potter, W.Z., Ritchie, G.F. and Rapoport, J.L. (1994) Cerebrospinal fluid monoamine metabolites in boys with attention-deficit hyperactivity disorder. *Psychiatry Res.*, 52: 305–316.
- Castellanos, F.X., Elia, J., Kruesi, M.J.P., Marsh, W.L., Gulotta, C.S., Potter, W.Z., Ritchie, G.F., Hamburger, S.D. and Rapoport, J.L. (1996) Cerebrospinal fluid homovanillic acid predicts behavioral response to stimulants in 45 boys with attention deficit/hyperactivity disorder. *Neuropsychopharmacology*, 14: 125–137.

- Cheetham, S.C., Viggers, J.A., Slater, N.A., Heal, D.J. and Buckett, W.R. (1993) [^3H] Paroxetine binding in rat frontal cortex strongly correlates with [^3H] 5HT uptake: effect of administration of various antidepressant treatments. *Neuropharmacology*, 32: 737–743.
- Committee on children and disabilities and committee on drugs. (1996) Medication for children with attentional disorders. *Pediatrics*, 98: 301–304.
- Consolo, S., Ramponi, S., Ladinsky, H. and Baldi, G. (1996) A critical role for D1 receptors in the 5-HT $_{1A}$ mediated facilitation of in vivo acetylcholine release in rat frontal cortex. *Brain Res.*, 707: 320–325.
- Curran, S., Purcell, S., Craig, I., Asherson, P. and Sham, P. (2005) The serotonin transporter gene as a QTL for ADHD. *Am. J. Med. Genet.*, 134B: 42–47.
- De Bruijn, E.R.A., Hulstijn, W., Verkes, R.J., Ruigt, G.S.F. and Sabbe, B.G.C. (2004) Drug-induced stimulation and suppression of action monitoring in healthy volunteers. *Psychopharmacology*, 177: 151–160.
- Dewey, S.L., Smith, G.S., Logan, J., Ding, Y.-S., King, P., Pappas, N.S., Brodie, J.D. and Ashby, C.R. (1995) Serotonergic modulation of striatal dopamine measured with positron emission tomography (PET) and in vivo microdialysis. *J. Neurosci.*, 15: 821–829.
- Diamond, A. (2007) Consequences of variations in genes that affect dopamine in prefrontal cortex. *Cereb. Cortex*, 17: i161–i170.
- Di Giovanni, G., Pierucci, M., Di Matteo, V. and Esposito, E. (2008) Serotonin/dopamine interactions: electrophysiological evidence. In: Di Giovanni, G. Di Matteo, V. and Esposito, E. (Eds.), *Serotonin–Dopamine Interaction: Experimental Evidence and Therapeutic Relevance*. Elsevier, Amsterdam, pp. 45–71.
- Di Mascio, M., Di Giovanni, G., Di Matteo, V., Prisco, S. and Esposito, E. (1998) Selective serotonin reuptake inhibitors reduce the spontaneous activity of dopaminergic neurons in the ventral tegmental area. *Brain. Res. Bull.*, 46: 547–554.
- Di Matteo, V., De Blasi, A., Di Giulio, C., and Esposito, E. (2001). Role of 5-HT(2C) receptors in the control of central dopamine function. *Trends Pharmacol Sci.* 22: 229–232.
- Doherty, M.D. and Pickel, V.M. (2001) Targeting of serotonin 1a receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain. *J. Comp. Neurol.*, 433: 390–400.
- Domschke, K., Sheehan, K., Lowe, N., Kirley, A., Mullins, C., O'Sullivan, R., Freitag, C., Becker, T., Conroy, J., Fitzgerald, M., Gill, M. and Hawi, Z. (2005) Association analysis of the monoamine oxidase A and B genes with attention deficit hyperactivity disorder (ADHD) in an Irish sample: preferential transmission of the MAO-A 941G allele to affected children. *Am. J. Med. Genet.*, 134B: 110–114.
- Donnelly, M., Zametkin, A.J., Rapoport, J.L., Ismond, D.R., Weingartner, H., Lane, E., Oliver, J., Linnoila, M. and Potter, W.Z. (1986) Treatment of childhood hyperactivity with desipramine: plasma drug concentration, cardiovascular effects, plasma and urinary catecholamine levels, and clinical response. *Clin. Pharmacol. Ther.*, 39: 72–81.
- Eisenberg, J., Mei-Tal, G., Steinberg, A., Tartakovsky, E., Zohar, A., Gritsenko, I., Nemanov, L. and Ebstein, R.P. (2003) Haplotype relative risk study of catechol-O-methyltransferase (COMT) and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity Val allele with ADHD impulsive-hyperactive phenotype. *Am. J. Med. Genet.*, 88: 497–502.
- Elfving, B., Madsen, J. and Knudsen, G.M. (2007) Neuroimaging of the serotonin reuptake site requires high-affinity ligands. *Synapse*, 61: 882–888.
- Ernst, M., Zametkin, A.J., Matochik, J.A., Jons, P.H. and Cohen, R.M. (1998) DOPA decarboxylase activity in attention deficit hyperactivity disorder adults. A [fluorine-18] fluorodopa positron emission tomography study. *J. Neurosci.*, 18: 5901–5907.
- Ernst, M., Zametkin, A.J., Matochik, J.A., Pascualvaca, D., Jons, P.H. and Cohen, R.M. (1999) High midbrain [18F]DOPA accumulation in children with attention deficit hyperactivity disorder. *Am. J. Psychiatry*, 156: 1209–1215.
- Fallgatter, A.J., Herrmann, M.J., Roemmler, J., Ehli, A.-C., Wagnen, A., Heidrich, A., Ortega, G., Zeng, Y. and Lesch, K.-P. (2005) Allelic variation of serotonin transporter function modulates the brain electrical response for error processing. *Neuropsychopharmacology*, 29: 1506–1511.
- Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A. and Sklar, P. (2005) Molecular genetics of attention deficit hyperactivity disorder. *Biol. Psychiatry*, 57: 1313–1323.
- Faraone, S.V., Sergeant, J.A., Gillberg, C. and Biederman, J. (2003) The worldwide prevalence of ADHD: is it an American condition? *World Psychiatry*, 2: 104–113.
- Ferrari, P.F., Van Erp, A.M., Tornatzky, W. and Miczek, K.A. (2003) Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *Eur. J. Neurosci.*, 17: 371–378.
- Findling, R.L., Greenhill, L.L., McNamara, N.K., Demeter, C.A., Kotler, L.A., O'Riordan, M.A., Myers, C. and Reed, M.D. (2007) Venlafaxine in the treatment of children and adolescents with attention-deficit/hyperactivity disorder. *J. Child. Adolesc. Psychopharmacol.*, 17: 433–445.
- Findling, R.L., Schwartz, M.A., Flannery, D.J. and Manos, M.J. (1996) Venlafaxine in adults with attention-deficit/hyperactivity disorder: an open clinical trial. *J. Clin. Psychiatry*, 58: 178–179.
- Floden, D., Alexander, M.P., Kubu, C.S. and Stuss, D.T. (2008) Impulsivity and risk-taking behavior in focal frontal lobe lesions. *Neuropsychologia*, 46: 213–223.
- Flory, J.D., Newcorn, J.H., Miller, C., Harty, S. and Halperin, J.M. (2007) Serotonergic function in children with attention-deficit hyperactivity disorder relationship to later antisocial personality disorder. *Br. J. Psychiatry*, 190: 410–414.
- Gainetdinov, R.R. and Caron, M.G. (2003) Monoamine transporters: from genes to behavior. *Ann. Rev. Pharmacol. Toxicol.*, 43: 261–284.
- Garcia-Cabezas, M.A., Rico, B., Sanchez-Gonzalez, M.A. and Cavada, C. (2007) Distribution of the dopamine innervation

- in the macaque and human thalamus. *Neuroimage*, 34: 965–984.
- Gastfriend, D.R., Biederman, J. and Jellinek, M.S. (1985) Desipramine in the treatment of attention deficit disorder in adolescents. *Psychopharmacol. Bull.*, 21: 144–145.
- Gogos, J.A., Morgan, M., Luine, V.N., Santha, M., Ogawa, S., Pfaff, D.W. and Karayiorgou, M. (1998) Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc. Natl. Acad. Sci. U.S.A.*, 95: 9991–9996.
- Goto, Y., Otani, S. and Grace, A.A. (2007) The Yin and Yang of dopamine release: a new perspective. *Neuropharmacology*, 53: 583–587.
- Gould, G.C., Altamirano, A.V., Javors, M.A. and Frazer, A. (2006) A comparison of the chronic treatment effects of venlafaxine and other antidepressants on serotonin and norepinephrine transporters. *Biol. Psychiatry*, 59: 408–414.
- Gualtieri, C.T. and Johnson, L.G. (2008) Medications do not necessarily normalize cognition in ADHD patients. *J. Atten. Disord.*, 11: 459–469.
- Guimaraes, A.P.M., Zeni, C., Polanczyk, G.V., Genro, J.P., Roman, T., Rohde, L.A. and Hutz, M.H. (2007) Serotonin genes and attention deficit/hyperactivity disorder in a Brazilian sample: preferential transmission of the HTR2A 452His allele to affected boys. *Am. J. Med. Genet. Part B*, 144B: 69–73.
- Haddjeri, N., Fare, C., Lucas, G., Mnie-Filali, O., Astier, B., Renaud, B., Blier, P. and Debonnel, G. (2004) In-vivo modulation of central 5-hydroxytryptamine (5-HT_{1A}) receptor-mediated responses by the cholinergic system. *Int. J. Neuropsychopharmacol.*, 7: 391–399.
- Hawi, Z., Dring, M., Kirley, A., Foley, D., Kent, L., Craddock, N., Asherson, P., Curran, S., Gould, A., Richards, S., Lawson, D., Pay, H., Turic, D., Langley, K., Owen, M., O'Donovan, M., Thapar, A., Fitzgerald, M. and Gill, M. (2002) Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT_{1B} receptor gene in 273 nuclear families from a multi-centre sample. *Mol. Psychiatry*, 7: 718–725.
- Hawi, Z., Foley, D., Kirley, A., McCarron, M., Fitzgerald, M. and Gill, M. (2001) Dopa decarboxylase gene polymorphisms and attention deficit hyperactivity disorder (ADHD): no evidence for association in the Irish population. *Mol. Psychiatry*, 6: 420–424.
- Hedges, D., Reimherr, F.W., Rogers, A., Strong, R. and Wender, P.H. (1995) An open trial of venlafaxine in adult patients with attention deficit hyperactivity disorder. *Psychopharmacol. Bull.*, 31: 779–783.
- Hesse, S., Ballaschke, O., Barthel, H., von Cramon, D.Y. and Sabri, O. (2006) The striatal dopamine transporter availability is reduced in adults with attention-deficit/hyperactivity disorder. *J. Nucl. Med.*, 47: p. 142P.
- Higley, J.D., King, S.T., Hasert, M.F., Champoux, M., Suomi, S.J. and Linnoila, M. (1996) Stability of interindividual differences in serotonin function and its relationship to severe aggression and competent social behavior in Rhesus Macaque females. *Neuropsychopharmacology*, 14: 67–76.
- Hirst, W.D., Cheung, N.Y., Rattay, M., Price, G.W. and Wilkin, G.P. (1998) Cultured astrocytes express messenger RNA for multiple serotonin receptor subtypes, without functional coupling of 5-HT₁ receptor subtypes to adenylyl cyclase. *Mol. Brain. Res.*, 61: 90–99.
- Iversen, S.D. and Iversen, L.L. (2007) Dopamine: 50 years in perspective. *Trends Neurosci.*, 30: 188–193.
- Jacobs, B.L. and Fornal, C.A. (1995) Serotonin and behavior: a general hypothesis. In: Bloom F.E. and Kupfer D.J. (Eds.), *Psychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 461–469.
- Jensen, P.S. and Arnold, L.E. (2004) National Institute of Mental Health Multimodal Treatment Study of ADHD follow-up: 24-month outcomes of treatment strategies for attention-deficit/hyperactivity disorder. *Pediatrics*, 113: 754–761.
- Kennedy, S.H., Konarski, J.Z., Segal, Z.V., Lau, M.A., Bieling, P.J., McIntyre, R.S. and Mayberg, H.S. (2007) Differences in brain glucose metabolism between responders to CBT and venlafaxine in a 16-week randomized controlled trial. *Am. J. Psychiatry*, 164: 778–788.
- Kent, L., Doerry, U., Hardy, E., Parmar, R., Gingell, K., Hawi, Z., Kirley, A., Lowe, N., Fitzgerald, M., Gill, M. and Craddock, N. (2002) Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): analysis and pooled analysis. *Mol. Psychiatry*, 7: 908–912.
- Kruesi, M.J.P., Rapoport, J.L., Hamburger, S.D., Hibbs, E., Potter, W.Z., Lenane, M. and Brown, G.L. (1990) Cerebrospinal fluid monoamine metabolites, aggression and impulsivity in disruptive behavior disorders of children and adolescents. *Arch. Gen. Psychiatry*, 47: 419–426.
- Krusch, D.A., Klorman, R., Brumaghim, J.T., Fitzpatrick, P.A., Borgstedt, A.D. and Strauss, J.S. (1996) Methylphenidate slows reactions of children with attention deficit disorder during and after an error. *J. Abnorm. Child Psychol.*, 24: 633–650.
- Leonard, B.E., McCartan, D., White, J. and King, D.J. (2004) Methylphenidate: a review of its neuropharmacological, neuropsychological and adverse clinical effects. *Hum. Psychopharmacol. Clin. Exp.*, 19: 151–180.
- Levesque, M. and Parent, A. (2005) The striatofugal fiber system in primates: a reevaluation of its organization based on single-axon tracing studies. *Proc. Natl. Acad. Sci. U.S.A.*, 102: 11888–11893.
- Levy, F. (1991) The dopamine theory of attention deficit hyperactivity disorder (ADHD). *Aust. N.Z. J. Psychiatry*, 25: 277–283.
- Levy, F. (2004) Synaptic gating and ADHD: a biological theory of comorbidity of ADHD and anxiety. *Neuropsychopharmacology*, 29: 1589–1596.
- Levy, F. and Hobbes, G. (1996) Does haloperidol block methylphenidate? Motivation or attention? *Psychopharmacology*, 126: 70–79.
- Li, J., Wang, Y., Zhou, R., Zhang, H., Yang, H., Yang, L., Wang, B. and Faraone, S.V. (2007) Association between polymorphisms in serotonin transporter gene and attention

- deficit hyperactivity disorder in Chinese Han subjects. *Am. J. Med. Genet. Part B*, 144B: 14–19.
- Loo, S.K., Specter, E., Smolen, A., Hopfer, C., Teale, P.D. and Reite, M.L. (2003) Functional effects of the DAT1 polymorphism on EEG measures in ADHD. *J. Am. Acad. Child. Adolesc. Psychiatry*, 42: 986–993.
- Luthman, J., Frederiksson, A., Sundström, E., Jonsson, G. and Archer, T. (1989) Selective lesion of central dopamine or noradrenaline neuron systems in the neonatal rat: motor behavior and monoamine alterations at adult stage. *Behav. Brain Res.*, 33: 267–277.
- Maidment, I.D. (2003) The use of antidepressants to treat attention deficit hyperactivity disorder in adults. *J. Psychopharmacol.*, 17: 332–336.
- Manor, I., Corbex, M., Eisenberg, J., Gritsenko, I., Bachner-Melman, R., Tyano, S. and Ebstein, R.P. (2004) Association of the dopamine D5 receptor with attention deficit hyperactivity disorder (ADHD) and scores on a continuous performance test (TOVA). *Am. J. Med. Genet.*, 127B: 73–77.
- Manor, I., Laibe, E., Eisenberg, J., Meidad, S., Lerer, E., Israel, S., Gritsenko, I., Tyano, S., Faraone, S.V. and Ebstein, R.P. (2008) Association between tryptophan hydroxylase 2 performance on a continuous performance test (T.O.V.A.) and response to methylphenidate in ADHD participants. *Am. J. Med. Genet. Part B*, DOI: 10.1002/ajmg.b.30702.
- Manor, I., Tyano, S., Eisenberg, J., Bachner-Melman, R., Kotler, M. and Ebstein, R.P. (2002) The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA). *Mol. Psychiatry*, 7: 790–794.
- Manuck, S.B., Bleil, M.E., Petersen, K.L., Flory, J.D., Mann, J.J., Ferrell, R.E. and Muldoon, M.F. (2005) The socioeconomic status of communities predicts variation in brain serotonergic responsivity. *Psychol. Med.*, 35: 519–528.
- Mateo, Y., Budygin, E.A., John, C.E. and Jones, S.R. (2004) Role of serotonin in cocaine effects in mice with reduced dopamine transporter function. *Proc. Natl. Acad. Sci. U.S.A.*, 101: 372–377.
- Mendlin, A., Martin, F.J. and Jacobs, B.L. (1999) Dopaminergic input is required for increases in serotonin output produced by behavioral activation: an in vivo microdialysis study in rat forebrain. *Neuroscience*, 93: 897–906.
- Martin-Ruiz, R., Ugedo, L., Honrubia, M.A., Mengod, G. and Artigas, F. (2001) Control of serotonergic neurons in rat brain by dopaminergic receptors outside the dorsal raphe nucleus. *J. Neurochem.*, 77(3): 762–775.
- Meneses, A. (1999) The 5-HT system and cognition. *Neurosci Biobehav. Rev.* 23: 1111–1125.
- Meyer, A. and Sagvolden, T. (2006) Fine motor skills in South African children with symptoms of ADHD: influence of subtype, gender, age, and hand dominance. *Behav. Brain Funct.*, 2: p. 33.
- Michelsen, K.A., Prickaerts, J. and Steinbusch, H.W.M. (2008) The dorsal raphe nucleus and serotonin – implications for neuroplasticity linked to major depression and Alzheimer's disease. In: Di Giovanni, G., Di Matteo, V., and Esposito, E. (Eds.), *Serotonin–Dopamine Interaction: Experimental Evidence and Therapeutic Relevance*.
- Michelsen, K.A., Schmitz, C. and Steinbusch, H.W.M. (2007) The dorsal raphe nucleus—from silver stainings to a role in depression. *Brain Res. Rev.*, 55: 329–342.
- Millan, M.J., Lejeune, F. and Gobert, A. (2007) Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *J. Psychopharmacol.*, 14: 114–138.
- Miyazaki, I., Asanuma, M., Diaz-Corralles, F.J., Miyoshi, K. and Ogawa, N. (2004) Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. *Brain Res.*, 1029: 120–123.
- Morrison, J.H. and Foote, S.L. (1986) Noradrenergic and serotonergic innervation of the cortical, thalamic, and tectal visual structures in old and new world monkeys. *J. Comp. Neurol.*, 243: 117–138.
- Myint, A.M., Kim, Y.K., Verkerk, R., Scharpe, S., Steinbusch, H.W.M. and Leonard, B.E. (2007) Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J. Affect Disord.*, 98: 143–151.
- Napier, T.C. and Istre, E.D. (2007) Methamphetamine-induced sensitization includes a functional upregulation of ventral pallidal 5-HT_{2A/2C} receptors. *Synapse*, 62: 14–21.
- Navailles, S., Moison, D., Cunningham, K.A. and Spampinato, U. (2008) Differential regulation of the mesoaccumbens dopamine circuit by serotonin_{2C} receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. *Neuropsychopharmacology*, 33: 237–246.
- Neale, B.M., Sham, P.C., Purcell, S., Banaschewski, T., Buitelaar, J., Franke, B., Sonuga-Barke, E.J.S., Ebstein, R.P., Eisenberg, J., Mulligan, A., Gill, M., Manor, I., Miranda, A., Mulas, F., Oades, R.D., Roeyers, H., Rothenberger, A., Sergeant, J.A., Steinhausen, H.-C., Taylor, E.A., Thompson, M., Zhou, K., Asherson, P. and Faraone, S.V. (2007) Population differences in the international multi-centre ADHD gene project. *Genet. Epidemiol.*, 32: 98–107.
- Nomura, M., Kusumi, I., Kaneko, M., Masui, T., Daiguji, M., Ueno, T., Koyama, T. and Nomura, Y. (2006) Involvement of a polymorphism in the 5-HT_{2A} receptor gene in impulsive behavior. *Psychopharmacology*, 187: 30–35.
- Nyman, E.S., Ogdie, M.N., Loukola, A., Varilo, T., Taanila, A., Hurtig, T., Moilanen, I.K., Loo, S.K., McGough, J.J., Järvelin, M.-R., Smalley, S.L., Nelson, S.F. and Peltonen, L. (2007) ADHD candidate gene study in a population-based birth cohort: association with DBH and DRD2. *J. Am. Acad. Child. Psychiatry*, 46: 1614–1621.
- Oades, R.D. (1985) The role of noradrenaline in tuning and dopamine in switching between signals in the CNS. *Neurosci. Biobehav. Rev.*, 9: 261–283.
- Oades, R.D. (1987) Attention deficit disorder with hyperactivity (ADHD): the contribution of catecholaminergic activity. *Prog. Neurobiol.*, 29: 365–391.
- Oades, R.D. (2002) Dopamine may be 'hyper' with respect to noradrenaline metabolism, but 'hypo' with respect to

- serotonin metabolism in children with ADHD. *Behav. Brain Res.*, 130: 97–101.
- Oades, R.D. (2005) The roles of norepinephrine and serotonin in ADHD. In: Gozal D. and Molfese D.L. (Eds.), *Attention Deficit Hyperactivity Disorder: From Genes to Animal Models to Patients*. Humana Press, Totowa, NY, pp. 97–130.
- Oades, R.D. (2006) Function and dysfunction of monoamine interactions in children and adolescents with AD/HD. In: Levin E.D. (Ed.), *Neurotransmitter Interactions and Cognitive Function*. Birkhäuser Verlag, Basel, pp. 207–244.
- Oades, R.D. (2007) The role of the serotonin system in ADHD: treatment implications. *Expert Rev. Neurother.*, 7: 1357–1374.
- Oades, R.D. and Halliday, G.M. (1987) The ventral tegmental (A 10) system. *Neurobiology I: anatomy and connectivity*. *Brain Res. Rev.*, 12: 117–165.
- Oades, R.D. and Müller, B.W. (1997) The development of conditioned blocking and monoamine metabolism in children with attention-deficit-hyperactivity disorder or complex tics and healthy controls: an exploratory analysis. *Behav. Brain Res.*, 88: 95–102.
- Oades, R.D., Slusarek, M., Velling, S. and Bondy, B. (2002) Serotonin platelet-transporter measures in childhood attention-deficit/hyperactivity disorder (ADHD): clinical versus experimental measures of impulsivity. *World J. Biol. Psychiatry*, 3: 96–100.
- O'Neill, M.F., Heron-Maxwell, C.L. and Shaw, G. (1999) 5-HT₂ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine and MK-801 but not D-1 agonist c-APB. *Pharmacol. Biochem. Behav.*, 63: 237–244.
- Overtoom, C.C.E., Verbaten, M.N., Kemner, C., Kenemans, J.L., van Engeland, H., Buitelaar, J.K., van der Molen, M.W., Van der Gugen, J., Westenburg, H.G.M., Maes, R.A.A. and Koelega, H.S. (2003) Effects of methylphenidate, desipramine and L-DOPA on attention and inhibition in children with attention deficit hyperactivity disorder. *Behav. Brain Res.*, 145: 7–15.
- Park, S.B., Coull, J.T., McShane, R.H., Young, A.H., Sahakian, B.J., Robbins, T.W. and Cowen, P.J. (1994) Tryptophan depletion in normal volunteers produces selective impairments in learning and memory. *Neuropharmacology*, 33: 575–588.
- Pelham, W.E. and Murphy, D.A. (1990) Attention deficit disorder. In: Pelham W.E. and Murphy D.A. (Eds.), *International Perspectives in Behavioral Medicine*. International Perspectives in Behavioral Medicine, Norwood, NJ, pp. 1–30.
- Phelix, C.F. and Broderick, P.A. (1995) Light microscopic immunocytochemical evidence for converging serotonin and dopamine terminals in ventrolateral nucleus accumbens. *Brain Res. Bull.*, 37: 37–41.
- Popper, C.W. (2000) Pharmacologic alternatives to psychostimulants for the treatment of attention-deficit/hyperactivity disorder. *Child Adolesc. Psychiatr. Clin. N. Am.*, 9: 605–646.
- Porras, G., Di Matteo, V., Fracasso, C., Lucas, G., De Deurwaerdere, P., Caccia, S., Esposito, E. and Spampinato, U. (2002) 5-HT(2A) and 5-HT(2C/2B) receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology*, 26: 311–324.
- Preuss, U.W., Koller, G., Bondy, B., Bahlmann, M. and Soyka, M. (2001) Impulsive traits and 5-HT_{2A} receptor promoter polymorphism in alcohol dependents: possible association but no influence of personality disorders. *Neuropsychobiology*, 43: 186–191.
- Qian, Q., Wang, Y., Zhou, R., Li, J., Wang, B., Glatt, S.J. and Faraone, S.V. (2003) Family-based and case-control association studies of catechol-O-methyltransferase in attention deficit hyperactivity disorder suggest genetic sexual dimorphism. *Am. J. Med. Genet. Part B*, 118: 103–109.
- Reuter, M., Kirsch, P. and Hennig, J. (2006) Inferring candidate genes for attention deficit hyperactivity disorder (ADHD) assessed by the World Health Organization Adult ADHD Self-Report Scale (ASRS). *J. Neural Transm.*, 113: 929–938.
- Reuter, M., Kuepper, Y. and Hennig, J. (2007) Association between a polymorphism in the promoter region of the *TPH2* gene and the personality trait of harm avoidance. *Int. J. Neuropsychopharmacol.*, 10: 401–404.
- Ribases, M., Ramos-Quiroga, J.A., Hervás, A., Bosch, R., Bielsa, A., Gastaminza, X., Artigas, J., Rodríguez-Ben, S., Estivill, X., Casas, M., Cormand, B. and Bayes, M. (2007) Exploration of 19 serotonergic candidate genes in adults and children with attention-deficit/hyperactivity disorder identifies association for 5HT_{2A}, DDC and MAOB. *Mol. Psychiatry*, doi:10.1038/sj.mp.4002100.
- Riikonen, R.S., Nokelainen, P., Valkonen, K., Kolemäinen, A.I., Kupulainen, K.I., Koenonen, M., Vanninen, R.-L.S. and Kuikka, J.T. (2005) Deep serotonergic and dopaminergic structures in fetal alcoholic syndrome: a study with nor-β-CIT-single-photon emission computed tomography and magnetic resonance imaging volumetry. *Biol. Psychiatry*, 57: 1565–1572.
- Roesch, M.R., Calu, D.J. and Schoenbaum, G. (2007) Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. *Nat. Neurosci.*, 10: 1615–1624.
- Rommelse, N.N.J., Altink, M.E., Oosterlaan, J., Buschgens, C.J.M., Buitelaar, J., de Sonnevle, L.M.J. and Sergeant, J.A. (2007) Motor control in children with ADHD and non-affected siblings: deficits most pronounced using the left hand. *J. Child. Psychol. Psychiatry*, 48: 1071–1079.
- Rosa-Neto, P., Lou, H.C., Cumming, P., Pryds, O., Karrebaek, H., Lunding, J. and Gjedde, A. (2005) Methylphenidate-evoked changes in striatal dopamine correlate with inattention and impulsivity in adolescents with attention deficit hyperactivity disorder. *Neuroimage*, 25: 868–876.
- Rubia, K., Lee, F., Cleare, A.J., Tunstall, N., Fu, C.H.Y., Brammer, M.J. and McGuire, P.K. (2004) Tryptophan depletion reduces right inferior prefrontal activation during no-go trials in fast, event-related fMRI. *Psychopharmacology*, 179: 791–803.
- Rubia, K., Smith, A.B. and Taylor, E.A. (2007) Performance of children with attention deficit hyperactivity disorder (ADHD) on a test battery of impulsiveness. *Child Neuropsychol.*, 13: 276–304.

- Russell, V.A., Oades, R.D., Tannock, R., Auerbach, J., Killeen, P.R., Johansen, E.B. and Sagvolden, T. (2006) Response variability in attention-deficit/hyperactivity disorder: a neuronal and glial energetics hypothesis. *BMC Behav. Brain Funct.*, 2: p. 30.
- Sagvolden, T., Johansen, E.B., Aase, H. and Russell, V.A. (2005) A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behav. Brain Sci.*, 28: 397–468.
- Schachar, R.J., Chen, S., Logan, G.D., Ornstein, T.J., Crosbie, J., Ickowicz, A. and Pakulak, A. (2004) Evidence for an error monitoring deficit in attention deficit hyperactivity disorder. *J. Abnorm. Child. Psychol.*, 32: 285–293.
- Scheres, A., Oosterlaan, J. and Sergeant, J.A. (2001) Response execution and inhibition in children with AD/HD and other disruptive disorders: the role of behavioural activation. *J. Child Psychol. Psychiatry*, 42: 347–357.
- Schmidt, L.A., Fox, N.A. and Hamer, D.H. (2007) Evidence for a gene-gene interaction in predicting children's behavior problems: association of serotonin transporter short and dopamine receptor D4 long genotypes with internalizing and externalizing behaviors in typically developing 7-year-olds. *Dev. Psychopathol.*, 19: 1105–1116.
- Schulz, K.P., McKay, K.E., Newcorn, J.H., Sharma, V., Gabriel, S. and Halperin, J.M. (1998) Serotonin function and risk for alcoholism in boys with attention-deficit hyperactivity disorder. *Neuropsychopharmacology*, 18: 10–17.
- Shaw, P., Eckstrand, K., Sharp, W., Blumenthal, J., Lerch, J.P., Greenstein, D., Clasen, L., Evans, A., Giedd, J.N. and Rapoport, J.L. (2007) Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. *Proc. Natl. Acad. Sci. U.S.A.*, 104: 19649–19654.
- Smoller, J.W., Biederman, J., Arbeitman, L., Doyle, A.E., Fagerness, J., Perlis, R.H., Sklar, P. and Faraone, S.V. (2006) Association between the 5HT1B receptor gene (*HTR1B*) and the inattentive subtype of ADHD. *Biol. Psychiatry*, 59: 460–467.
- Snoek, H., van Goozen, S.H.M., Matthys, W., Sigling, H.O., Koppeschaar, H.P.F., Westenberg, H.G.M. and van Engeland, H. (2002) Serotonergic functioning in children with oppositional defiant disorder: a sumatriptan challenge study. *Biol. Psychiatry*, 51: 319–325.
- Sonuga-Barke, E.J.S. (2005) Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. *Biol. Psychiatry*, 57: 1231–1238.
- Stadler, C., Schmeck, K., Nowraty, I., Müller, W.E. and Poustka, F. (2004) Platelet 5-HT uptake in boys with conduct disorder. *Neuropsychobiology*, 50: 244–251.
- Stoff, D.M., Pollock, L., Vitiello, B., Behar, D. and Bridger, W.H. (1987) Reduction of (3H)-imipramine binding sites on platelets of conduct-disordered children. *Neuropsychopharmacology*, 1: 55–62.
- Swanson, J.M., Kinsbourne, M., Nigg, J.T., Lanphear, B., Stefanotos, G.A., Volkow, N.D., Taylor, E.A., Casey, B.J., Castellanos, F.X. and Wadhwa, P.D. (2007) Etiologic subtypes of attention-deficit/hyperactivity disorder: Brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol. Rev.*, 17: 39–59.
- Telang, F.W., Volkow, N.D., Levy, A., Logan, J., Wong, C. and Wang, G.J. (1999) Distribution of tracer levels of cocaine in the human brain as assessed with averaged [11C]cocaine images. *Synapse*, 31: 290–296.
- Todd, R.D. and Botteron, K.N. (2001) Is attention-deficit/hyperactivity disorder an energy deficiency syndrome? *Biol. Psychiatry*, 50: 151–158.
- Tucha, O., Mecklinger, L., Laufkoetter, R., Kaunzinger, I., Paul, G.M., Klein, H.E. and Lange, K.W. (2005) Clustering and switching on verbal and figural fluency functions in adults with attention deficit hyperactivity disorder. *Cogn. Neuropsychiatry*, 10: 231–248.
- Uehara, T., Sumiyoshi, T., Matsuoka, T., Itoh, H. and Kurachi, M. (2006) Role of 5-HT_{1A} receptors in the modulation of stress-induced lactate metabolism in the medial prefrontal cortex and basolateral amygdala. *Psychopharmacology*, 186: 218–225.
- Uzbekov, M.G. (2006) Hyperkinetic syndrome as a manifestation of a disturbance of metabolism and mental development. In: Oades R.D. (Ed.), *Attention-Deficit/Hyperactivity Disorder and the Hyperkinetic Syndrome: Current Ideas and Ways Forward*. Nova Science Publishers, Inc., Hauppauge, NY, pp. 133–154.
- van der Plasse, G. and Feenstra, M.G.P. (2007) Serial reversal learning and acute tryptophan depletion. *Behav. Brain Res.*, 186: 23–31.
- van Goozen, S.H.M., Fairchild, G., Snoek, H. and Harold, G.T. (2007) The evidence for a neurobiological model of childhood antisocial behavior. *Psychol. Bull.*, 133: 149–182.
- Van Meel, C.S., Heslenfeld, D.J., Oosterlaan, J. and Sergeant, J.A. (2007) Adaptive control deficits in attention-deficit/hyperactivity disorder (ADHD): the role of error processing. *Psychiatry Res.*, 151: 211–220.
- Vertes, R.P. (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *J. Comp. Neurol.*, 313: 643–668.
- Volkow, N.D., Wang, G.-J., Newcorn, J.H., Fowler, J.S., Telang, F.W., Solanto, M.V., Logan, J., Wong, C., Ma, Y., Swanson, J.M., Schulz, K.P. and Pradhan, K. (2007a) Brain dopamine transporter levels in treatment and drug naïve adults with ADHD. *Neuroimage*, 34: 1182–1190.
- Volkow, N.D., Wang, G.-J., Newcorn, J.H., Telang, F.W., Solanto, M.V., Fowler, J.S., Logan, J., Ma, Y., Schulz, K., Pradhan, K., Wong, C. and Swanson, J.M. (2007b) Depressed dopamine activity in caudate and preliminary evidence of limbic involvement in adults with attention-deficit/hyperactivity disorder. *Arch. Gen. Psychiatry*, 64: 932–940.
- Vollmar, P., Haghighi, A., Dermietzel, R. and Faustmann, P.M. (2007) Venlafaxine exhibits an anti-inflammatory effect in an inflammatory co-culture model. *Int. J. Neuropsychopharmacol.*, 11: 111–117.
- Walderhaug, E., Lunde, H., Nordvik, J.E., Landro, N.I., Refsum, H. and Magnusson, A. (2002) Lowering of serotonin by rapid tryptophan depletion increases impulsiveness in normal individuals. *Psychopharmacology*, 164: 385–391.

- Weikop, P., Kehr, J. and Scheel-Kruger, J. (2007a) Reciprocal effects of combined administration of serotonin, noradrenaline and dopamine reuptake inhibitors on serotonin and dopamine levels in the rat prefrontal cortex: the role of 5-HT1A receptors. *J. Psychopharmacol.*, 21: 795–804.
- Weikop, P., Yoshitake, T. and Kehr, J. (2007b) Differential effects of adjunctive methylphenidate and citalopram on extracellular levels of serotonin, noradrenaline and dopamine in the rat brain. *Eur. Neuropsychopharmacol.*, 17: 658–671.
- Wiersma, J.R., van der Meere, J.J. and Roeyers, H. (2005) ERP correlates of impaired error monitoring in children with ADHD. *J. Neural. Transm.*, 112: 1417–1430.
- Wigal, S.B., Swanson, J.M., Feifel, D., Sangal, R.B., Elia, J., Casat, C.D., Zeldis, J.B. and Conners, C.K. (2004) A double-blind, placebo-controlled trial of dexamethylphenidate hydrochloride and d,l-threo-methylphenidate hydrochloride in children with attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry*, 43: 1406–1414.
- Wigg, K.G., Takhar, A., Ickowicz, A., Tannock, R., Kennedy, J.L., Pathare, T., Malone, M., Schachar, R.J. and Barr, C.L. (2006) Gene for the serotonin transporter and ADHD: no association with two functional polymorphisms. *Am. J. Med. Genet. Part B*, 141B: 566–570.
- Winstanley, C.A., Theobald, D.E.H., Dalley, J.W., Cardinal, R.N. and Robbins, T.W. (2006) Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cereb. Cortex*, 16: 106–114.
- Zametkin, A.J. and Rapoport, J.L. (1987) Neurobiology of attention deficit disorder with hyperactivity: where have we come in 50 years? *J. Am. Acad. Child. Psychiatry*, 26: 676–686.

CHAPTER 27

Serotonin/dopamine interaction in learning

María Esther Olvera-Cortés^{1,3,*}, Patricia Anguiano-Rodríguez¹, Miguel Ángel López-Vázquez² and José Miguel Cervantes Alfaro³

¹*Laboratorio de Neurofisiología Experimental, Centro de Investigación Biomédica de Michoacán, Instituto Mexicano del Seguro Social, Morelia, Mich., México*

²*Laboratorio de Neuroplasticidad de los Procesos Cognitivos, Centro de Investigación Biomédica de Michoacán, Instituto Mexicano del Seguro Social, Morelia, Mich., México*

³*Laboratorio de Neurociencias, División de Estudios de Posgrado, Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana de San Nicolás de Hidalgo, Morelia Mich., México*

Abstract: Dopamine (DA)–serotonin interactions dealing with learning and memory functions have been apparent from experimental approaches over the past decade. However, since the former evidence showing that these cerebral neurotransmitter systems are involved in the regulation of the same cognitive processes, few experimental studies have been done to further clarify the nature of DA–serotonin interactions for cognitive processes sharing common brain structures. Nevertheless, a regulatory role of 5-HT/DA interactions in cognition and the prefrontal cortex (PFC) and the striatum as a neuroanatomical substrate for these DA/5-HT interactions, are now recognized. Experimental evidence indicates that pharmacological disruption of serotonin neurotransmission results in a facilitative effect on the processing of mnemonic information by cerebral regions under strong, functional DA modulation, such as the striatum and the PFC; on the other hand, increased serotonin neurotransmission appears to have a detrimental effect on cognitive functions integrated in these structures. These effects seem to occur through the interaction of different pre- and postsynaptic DA and serotonin receptor subtypes acting as opposite systems underlying cognitive abilities. Some studies, focused on DA–serotonin interactions underlying the pathophysiology of neurological and psychiatric diseases, which evolve with cognitive dysfunctions in human beings, have shown that drugs that are able to modify DA or serotonin neurotransmission may exert beneficial effects on cognitive functions, even though improvement of motor, mood and behavioural disturbances are the main objectives of pharmacological treatment of these diseases. The complete significance of DA–serotonin interactions in cognitive functions could be addressed by future experimental and clinical studies.

Keywords: serotonin; cognition; striatum; prefrontal cortex; Parkinson's disease; dopamine receptors; learning

Introduction

Over recent decades, there has been mounting evidence for the participation of a number of

different hormonal and neurotransmitter systems in cognitive processes. However, interaction between neurotransmitters in the performance of cognitive tasks is a less developed issue. It has been established that many of the complex actions of the central nervous system (CNS) are determined by the properties of particular neurotransmitter

*Corresponding author. Tel.: +52 4433 241610;
Fax: +52 4433 241610; E-mail: maesolco@yahoo.com

systems, and by the interactions between them (Trimmer, 1999). Neuromodulation is a term that consistently describes non-classical effects of neurotransmitters on neurons. Thus, neuromodulation occurs when a substance released from one neuron alters the cellular or synaptic properties of another neuron (Kupfermann, 1979; Kaczmarek and Levitan, 1987). There are centres in the brain responsible for producing neuromodulatory effects and between these centres are the raphe nuclei (RN) and substantia nigra (SN), which are small clusters of neurons located in the brain stem with diffuse projections to all other areas of the brain. Their divergent projection pattern suggests that these neurons modulate activity in other areas of the brain, and practically all neuronal circuits in the mammalian brain are subject to neuromodulation arising from these centres (Katz, 1999).

There are three major dopaminergic (DAergic) systems in the brain (Wolf et al., 1987): the mesostriatal system originating in the SN pars compacta, the mesolimbic system originating in the ventral tegmental area (VTA) and terminating in the accumbens nucleus (AN) and the mesocortical system originating in the VTA, but terminating in the prefrontal cortex (PFC). The cell bodies and terminal regions of all three DAergic pathways are innervated by serotonergic (5-HTergic) neurons originating in the medial and dorsal raphe nucleus (DRN) (Geyer et al., 1976; Parent et al., 1981; Beart and McDonald, 1982; Nedergaard et al., 1988). Neurons in the DRN make connections with areas innervated by the DAergic system (amygdala, striatum and PFC), whereas medial raphe nucleus (MRN) neurons make connections to the hippocampus and septal nuclei, which are not major DAergic targets (Azmitia and Segal, 1978). The dopamine (DA) and serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system activities are independently related to each other in the modulation of diverse cognitive abilities. However, little is known about the interaction between these two systems in the modulation of cognition. Nevertheless, a neuro-anatomical substrate for DA/5-HT interaction exists, and the distribution of DA and 5-HT receptors allows us to deduce the possible interactions between these neurotransmission systems.

We will emphasize the DA/5-HT interactions in those cerebral regions implicated in cognition that receive both DA and 5-HT innervation, and where it has been shown that the participation of these neurotransmitters causes modulation of cognitive performance. For this reason, the focus of the present chapter will analyse the participation of 5-HT and DA in cognition separately and then, we will analyse the mesostriatal and mesocortical target areas, their participation in cognitive processes and the regulatory role of 5-HT/DA interactions in cognition sustained by processes principally underlying the PFC and striatum.

The serotonergic system and cognition

Serotonergic systems have been implicated in diverse behavioural processes including motor function, motivation, timing behaviour, behavioural inhibition and response to stress and threat (Iversen, 1984; Fletcher et al., 1999; Misane and Ögren, 2000). Experimental work over recent decades has led to the general notion that experimental pharmacological and neurotoxic manipulations that reduce central 5-HTergic transmission increase the retention, or facilitate the acquisition, of information, whereas manipulations that increase the 5-HTergic function produce deficiencies in learning and retention of certain tasks (Asin and Fibiger, 1984; Altman et al., 1990; Fletcher et al., 1999).

Cerebral 5-HT manipulation

Early experimental works studied the effect of cerebral 5-HTergic depletion on diverse learning and memory abilities. The principal strategies used to deplete brain 5-HT were the intracerebroventricular (ICV) or intra raphe application of 5,7-dihydroxytryptamine (5,7-DHT) and the intraperitoneal application of *para*-chlorophenylalanine (PCPA). Early results indicated that 5-HT depletion produced impairments, improved or did not affect memory and learning, depending on pharmacological approach, doses and behavioural tests used, and depending on what type of information was used by the animals and the nature of the

associations established. Regarding human studies, the principal strategy used consisted of the modification of cerebral 5-HT levels through the manipulation of tryptophan (Trp) availability, because this essential amino acid is the precursor of 5-HT synthesis, and it has been extensively shown that modifications in dietary availability of Trp causes modifications in cerebral 5-HT content (Biggio et al., 1974). However, a recent study directed at validating the use of acute Trp depletion (ATD) to effect a reduction in 5-HT and DA efflux, found that despite the plasma reduction being similar to that reported after experimental ATD, no effect on efflux of DA and 5-HT was observed in the PFC (Plasse et al., 2007). Thus, behavioural effects observed after ATD must be interpreted cautiously, because they could be the result of compensatory changes in synaptic process and neurotransmitter systems, rather than being directly related to a reduction of serotonergic function.

We will now provide a summary of experimental findings related to serotonergic manipulation and its effects on cognitive ability, including a limited number of works that show general outcomes obtained through similar work, and some relevant aspects will be highlighted. Frequently in cognitive tasks, individual processes are intermixed, for example, working memory is not dissociated from spatial processing, and short-term memory (STM) is not well-differentiated from working memory. Thus, neurotransmitter participation in the processing of different information can be interpreted from a single experiment, and for this reason, an experimental finding can be relevant to more than one cognitive process.

Early works indicated that a behavioural test implicating spatial management was not affected by cerebral or hippocampal 5-HT depletion. Experimental ICV 5,7-DHT application producing cerebral 5-HT depletion had no effect on the performance of rats in a radial arm test or in a water maze (Richter-Levin and Segal, 1989; Altman et al., 1990; Murtha and Pappas, 1994), whereas this same pharmacological strategy resulted in better performance in active avoidance tests (Carli et al., 1995). In more recent work, it was reported that the ICV injection of 5,7-DHT (150 µg in 4.5 µl/ventricle) significantly diminished

spontaneous alternation in a Y-maze when the reduction of 5-HT in the PFC was about 85% (Hritcu et al., 2007). This could be related to the effect of increased perseveration reported after prefrontal 5-HT, because several findings indicate that 5-HT is more involved in the reversal of learning ability than working memory *per se*, and that prefrontal 5-HT depletion results in a deficiency in reversal learning, but only in the reversion of acquired rules without affecting working memory (Robbins and Roberts, 2007).

Whereas it has been shown that 5-HT depletion alone does not cause alterations in place of learning ability, a double lesion of acetylcholine and 5-HT caused a more profound deficit than that observed after cholinergic lesions alone (Richter-Levin and Segal, 1989). However, a deficit in reference spatial memory assessed in a water maze, spatial working memory assessed in radial arm maze and reduction in spontaneous alternation in T-maze produced by cholinergic denervation induced by the intraseptal application of 192IgG-saporin were attenuated by hippocampal 5-HT depletion through injections of 5,7-DHT into the fimbria fornix and cingulate bundle, despite hippocampal 5-HT depletion alone (about 55% depletion) not affecting the performance of any test (Lehmann et al., 2002). Thus, 5-HT depletion can reverse cognitive deficits produced by acetylcholine depletion. However, in other cognitive tests, 5-HT depletion resulted in a facilitation of performance. For example, 5-HT depletion after application of *p*-chloroamphetamine (PCA) alone, or combined with bilateral ibotenic acid-mediated lesions of nucleus basalis magnocellularis (NBM), was induced in rats and later evaluated in a 14-unit Stone maze, which was a complex, positively reinforced spatial discrimination task. Cerebral serotonergic depletion produced enhanced learning in the task that was completely prevented by a basal magnocellularis nucleus lesion (Normile et al., 1990), implicating a 5-HT/cholinergic interaction. The same investigators (Altman et al., 1990) trained rats in the 14-unit Stone T-maze after selective hippocampal 5-HT depletion induced by deafferentation with 5,7-DHT infused into the fimbria fornix and cingulate bundle. The lesioned rats reached the learning

criteria in significantly fewer trials, than the control rats did, and made significantly fewer errors throughout the training. Thus, both cerebral or hippocampal 5-HT depletion resulted in facilitation in this spatial tests. However, considering that the hypothesis regarding the participation of different memory systems in place- or egocentric-learning that could have cooperative or competitive interactions (McDonald and White, 1993), and because the Stone test has a strong egocentric component, raises questions regarding whether the egocentric component of the task influences the better performance.

Moreover, STM was evaluated using Trp-restricted rats using Biel's maze, which consists of a series of T disjunctions with a longer final corridor in which the animal finds a reward, and the animals had to choose the correct pathway based on left–right turns, but could also make use of spatial information. Using this task, González-Burgos et al. (1998) observed a facilitation of STM, which was expressed as an early significant reduction in errors made by the animals during single-session training, such that the animals reduced the number of errors made after the second training trial compared to control animals that made it to the fourth trial. The same authors performed an evaluation of STM after prefrontal 5-HT depletion through application of 5,7-DHT into DRN (1 µg/1 µl), and observed the same facilitatory effect in the performance of the rats (Pérez-Vega et al., 2000).

However, more recently, it has been reported that ICV injection of 5,7-DHT (150 µg in 4.5 µl per ventricle) produced deficiencies in working memory in a radial arm maze, which was interpreted as a result of a deficiency in STM, because no effects were observed in reference memory in a radial arm tests (Hritcu et al., 2007). A test of the step-through latency in a multi-trial passive avoidance (PA) task was used to evaluate long-term memory (LTM), and no effect was observed after 5-HT. Moreover, the administration of PCPA neither altered the PA performance. The investigators only measured 5-HT depletion in the PFC, and observed a depletion of approximately 85% after 5,7-DHT treatment and 80% after PCPA treatment (Hritcu et al., 2007). An important difference between these

two experimental approaches is the use of different behavioural tests. Biel's maze evaluates STM (not working memory), and poses a strong egocentric component, in such a manner that to solve this test the animals can make use of a series of left–right turns without using spatial information, or they can use a spatial mapping strategy, as required for radial arm maze resolution.

To assess the possibility that an egocentric component of behavioural tests could account for the facilitating effect observed after 5-HT depletion, spatial egocentric learning ability was tested to cerebral 5-HT depleted rats using a Morris water maze. The rats received a unique intracisternal injection of 5,7-DHT at 21-days old, and were evaluated in a Morris maze at 60-days old, using a behavioural task designed to avoid the use of allocentric spatial cues to solve the Morris maze task. With this aim, a black curtain was used to surround the maze, and the starting point and the platform position, although relatively constant, were rotated in the maze. Cerebral 5-HT depletion produced a facilitatory effect on egocentric learning, which was evident in reduced escape latencies. The performance of experimental rats on the first day of training and in all six training days was compared with the control animals that were unable to learn the task (Olvera-Cortés et al., 2001).

From the experimental findings described above, the proposition that the nature of the information used by experimental subjects is an important determinant of the consequences of 5-HT manipulations emerges, in a manner that suggests that spatial information processing is not affected by 5-HT depletion, but rather, the processing of egocentric information (or tests with a strong egocentric component) is favoured by 5-HT depletion. McDonald and White (1993) proposed a dissociation between memory systems in which the hippocampus forms part of a system engaged in the processing of stimulus–stimulus associations (or configural associations), such as those used in the establishment of cognitive maps, whereas stimulus–response associations (such as those used in egocentric learning) are directed by the activity of a memory system, including the striatum as its principal component. Cooperative and competitive interactions can occur between

these systems. From this perspective, if egocentric processing, which implies striatal activity, is favoured by 5-HT depletion, other striatal-dependent cognitive processes must show a facilitating effect after 5-HT depletion.

Conditioned tests involve the acquisition of stimulus–response associations, and have been extensively shown to require the participation of striatal activity (Kirkby and Polgar, 1974; McDonald and White, 1993). The effect of a reduction in 5-HT after the application of 5,7-DHT in DRN and MRN in thirsty rats trained to associate a conditioned stimulus with water delivery were tested by Fletcher et al. (1999). A reduction in 5-HT, both in the hippocampus and striatum, was associated with an enhancement in the conditioned response, so the authors suggested that a reduction in 5-HT removes the inhibitory influence on the mesolimbic DArgic system, resulting in an increase in conditioned responses (Fletcher et al., 1999). However, 5-HT depletion in the neocortex, hippocampus and striatum of more than 90%, induced by the ICV application of 5,7-DHT in rats, led to the 5-HT depleted animals failing to acquire conditional visual discrimination in a go/no-go task. This deficiency was probably due to an inability to withhold responses, and thus, correctly complete the no-go trials. The depleted rats responded faster, but correctly, during the go trials, and incorrectly during the no-go trials (Harrison et al., 1999). Thus, the possibility exists that impulsivity can account for the errors in the no-go trials. However, when the investigators used trained rats with their conditioning established, and then effected 5-HT depletion, these animals showed a similar, although less severe effect, on the performance in the no-go trials, showing an increase in response. Therefore, although the animals were able to perform discriminative learning before the lesions, 5-HT depletion produced a more deficient response during the no-go trials associated with an increased impulsivity more than the response to acquisition deficiencies (Harrison et al., 1999). However, forebrain 5-HT depletion after ICV 5,7-DHT administration caused a facilitation in acquisition of the discrimination that occurred earlier than in the controls in a task involving simple conditional visual

discrimination. A similar facilitating effect was observed in rats after the infusion of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) into the RN (reducing 5-HT release through 5-HT_{1A} autoreceptors) (Ward et al., 1999). Ward et al. (1999) evaluated the acquisition and performance of conditional visual discrimination after forebrain 5-HT depletion in a task requiring the acquisition of a ‘stimulus–response’ rule or habit (Mishkin et al., 1984). The animals were required to learn the following rules to obtain a sucrose solution: ‘if fast go left’ and ‘if slow go right’ (‘fast’ corresponded to 0.1 s light pulses at a frequency of 5 Hz, and ‘slow’ corresponded to light pulses with a duration of 2.4 s at a frequency of 0.83 Hz). ICV infusion of 5,7-DHT was made in order to the lesion 5-HTergic cells, whereas cannulation of the RN was performed to infuse the 5-HT₁ receptor agonist 8-OH-DPAT into the dorsal raphe of other group of rats. The rats injected with 5,7-DHT had reductions in 5-HT in the hippocampus and cortex (80%), as well as in the striatum (greater than 90%), with a less substantial reduction in the hypothalamus. The 5-HT depletion did not affect post-operative re-acquisition of lever pressing under a continuous reinforcement schedule, but in the visual discrimination task, the depleted rats reached the most stringent criteria of acquisition (85%) in fewer sessions than the sham animals did, although the facilitation occurred in the first part of the acquisition (when the criterion was moved from 59% to 67%). 5-HT-depleted rats also showed a tendency to make fewer errors of commission. Thus, in no-go tasks, 5-HT depletion caused a deficiency attributed to a perseverative response, but in this work, two rules implying a go-task were favoured by 5-HT depletion. Together, these findings indicate that 5-HT facilitates conditioned visual discrimination, whereas it produces deficiencies in tasks that include a component of inhibitory response, as found in no-go trials. Moreover, the effect of systemic PCA application on the performance of a one-way active avoidance task by rats was examined, and it was observed that an increase in 5-HT produced impairments in the acquisition and retention of a task, depending on the temporal effects of the drug on 5-HT release. The effect was blocked by the

application of PCPA, but not by catecholamine depletion (through α -methyl-*p*-tyrosine or by application of the selective noradrenergic toxin, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP4)) (Ögren, 1985). Moreover, the systemic application of the 5-HT receptor agonist 8-OH-DPAT reduced the escape latency in PA tests (Misane and Ögren, 2002). Findings such as these support a role for 5-HT in the regulation of aversive learning, and the view that 5-HT regulation predominates in cognitive processes organized by cortico-striatal activity, which is relevant, because of the preponderant participation of DA in regulation of these cerebral structures.

Table 1 summarizes the principal findings described, and highlights important insights obtained from the data. Firstly, the behavioural tests that possess egocentric components are favoured by 5-HT depletion. Secondly, conditioning tasks, such as PA or instrumental tasks, are frequently favoured by 5-HT depletion (with the exception of no-go tests in which a component of impulsivity is primarily related to deficient learning). Thirdly, in general, these tasks are dependent on striatal function (or cortico-striatal processing) as part of a memory system that is under strong

DArgic modulation and is also extensively innervated by 5-HTergic terminals.

Electrophysiological studies have demonstrated that systemic or local application of 5-HT receptor agonists and antagonists onto DArgic neurons in the VTA or SN modulate the spontaneous activity of DArgic neurons (Kelland et al., 1990). 5-HT normally inhibits the cell firing of DArgic nigral neurons, and thereby, may modulate DA-dependent cognitive processes. A review of prominent findings regarding DA regulation of cognition will allow us to see an integrative view of the roles of 5-HT and DA in cognition.

The dopaminergic system and cognition

DA plays a significant role in memory processes, especially through the interconnection of two brain regions: the striatum and the PFC (Jay, 2003). Extensive clinical and experimental findings involve the DArgic system with working memory organization. Parkinson's disease (PD) patients, whose diminished DA release in basal ganglia caused by DArgic nigral cell death, presents a deficit in working memory that is attenuated by

Table 1. Behavioural consequence of serotonergic modifications on learning and memory tests in rat studies

Pharmacological approach	Administration via/effect	Spatial learning	STM/LTM	Cognitive process			
				Working memory	Spontaneous alternation	Avoidance	Conditioning
5,7-DHT	ICV/CD	WMPL =	RAM =/=	RAM–	TM–	PA +	S +
	HC-FF/HD WMPL = SM + IC/CD Raphe/CD Raphe/PFCD	WMEL +	BM +/	DeA–	YM–		Go + /no-go–
8-OHDPAT	Raphe/CD						Go +
Trp	Diet restriction/ CD				TM (+)		
	Ip./CI		+ / +				
PCA	Ip./CD	SM +				AA–	
PCPA	Ip./CD	WMCL–					
Fluoxetine	Ip./CI			DNMPT–			

Symbols: =, no changes; +, facilitation effect; –, detrimental effect. Abbreviations: CD, cerebral depletion; CI, cerebral increase; HD, hippocampal depletion; PFCD, prefrontal cortex depletion; ICV, intracerebroventricular; HIPP, hippocampal; BM, Biel's maze; WM, water maze; RAM, radial arm maze; ST, Stone's maze; TM, T maze; YM, Y maze; DeA, delayed alternation; DNMPT, delayed non-matching to position tests; S, simple conditioning; Go, Go tests; No-go, no-go tests; PA, passive avoidance; AA, active avoidance; WMCL, water maze cue learning; WMEL, water maze egocentric learning. Chemical compounds, see abbreviations list.

levodopa (L-dopa) administration (Lewis et al., 2005). Similarly, DA loss in the PFC causes profound working memory deficit in monkeys and rats (Simon et al., 1980). Experimentally, exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 30 mg/kg \pm 3 c/12 h, i.p.) produced a depletion of 60% in striatal DA in rats. The performance of animals with this degree of DA reduction in a T maze delayed alternation test was impaired, but only under conditions of delayed response (20 s and 120 s), and this is indicative of spatial working memory impairment (Tanila et al., 1998). This effect was apparently mediated by the effect of DA depletion on the PFC, because similar results were obtained after specific prefrontal DA depletion induced by 6-hydroxydopamine (6-OH-DA) without causing 5-HTergic or catecholaminergic alterations. Using this approach, Bubser and Schmidt (1990) observed deficiencies in a T maze-delayed alternation task and deficiencies in a radial arm maze, whereas in continuous tasks, no deficiencies were observed. However, the PFC and the striatum work as a system in the organization of the different aspects of working memory. For example, it has been reported that in mice, a transient increase in D₂ receptor expression restricted to the striatum causes a selective cognitive impairment in a working memory task, and a behavioural flexibility that was associated with alterations in DArgic function in the PFC, including DA turnover and the activity of D₁ prefrontal receptors (Kellendonk et al., 2006).

Improvement in spatial working memory has been observed after administration of pergolide, a mixed agonist of D₁ and D₂ DA receptors, to healthy subjects evaluated using a visuospatial-delayed matching test, whereas bromocriptine, a D₂ receptor agonist, did not have an effect on the same task (Müller et al., 1998). DA participation in working memory organization is well-documented, and implies DA actions, principally in the PFC.

Experimental findings have implicated DA as a major participant in brain-reward circuitry, not as a mediator of the hedonic aspect of reward, but rather being involved in anticipatory aspects of rewards and the incentive value for motivated behaviours (Salomone et al., 1997; Ikemoto and

Panksepp, 1999; Wightman and Robinson, 2002). According to Ikemoto and Panksepp (1999), 'Incentive motivation is a process in which approach or avoidance responses are generated by stimuli that predict the proximity or availability of unconditioned stimuli (positive or negative)'. When a reward is presented to a monkey, its DArgic neurons increase their firing rate within a period of a few milliseconds of delivery of the reward. If the animals are trained to anticipate the reward, pairing its presentation with a conditional cue, then the DArgic neurons respond to the cue more than to the reward itself (Mireniewicz and Schultz, 1994). Schultz (1998) proposed that these phasic changes in DArgic cell activity, which have been associated with large and transient increases in extracellular DA levels in terminal regions, could interact with remote receptors and act as an alerting signal for salient stimuli (Rebec et al., 1997; Robinson et al., 2001). Slower tonic changes in DA may regulate other aspects of reward (Wightman and Robinson, 2002). Recently, Pizzagalli et al. (2008) administered healthy volunteers with low doses of the D₂/D₃ receptor agonist pramipexole (0.5 mg/kg) to decrease the phasic DA release through actions on autoreceptors. The subjects showed a decreased response to a rewarded stimulus, which was independent of motor adverse effects, confirming that in humans, phasic DA release is required to reinforce actions (stimulus-response establishment) leading to reward. It is important to recapitulate at this point that 5-HT depletion is related to enhancement of conditioned responses, because of the probable relevance of DA in conditioning 5-HT/DA interactions in the organization of conditioning. In this sense, a hypothesis developed by Daw et al. (2002) based on indirect evidence, places 5-HT acting as an aversive system in opposition to a DA system acting as an appetitive system, constituting motivational opponency.

DA depletion also participates in the organization of procedural learning, and evidence had been obtained from tests of motor sequential learning that depend on the integrity of corticostriatal functioning (Tinaz et al., 2006), and are also dependent on the DArgic system (Matsumoto et al., 1999; Carbon et al., 2004; Badgaiyan

et al., 2007). Impairment in sequential motor learning, evaluated in a radial arm maze, has been observed as a general consequence of monoamine depletion, and is particularly related to the degree of DA depletion in the dorsal striatum (Daberkow et al., 2005). In humans, sequential learning is impaired in DA-deficient patients (Carbon et al., 2004). Badgaiyan et al. (2007) used a dynamic molecular imaging technique that located regions where the DA receptor ligand ^{11}C -raclopride was displaced from receptors by DA released endogenously during the performance of a sequential motor learning task. Their findings indicate DA release from the posterior two-thirds of the dorsomedial aspect of the putamen and the caudate anterior, suggesting that striatal DArgic mechanisms are involved in human sequential learning. Similarly, in experimental studies, it has been observed that the striatum is a key nucleus in cognitive functions underlying basal ganglia activity (Matsumoto et al., 1999). For example, lesions or inactivation of striatum impairs the acquisition of instrumental tasks, stimulus–response learning and temporal expectation in rats (McDonald and White, 1993; Florio et al., 1999; Hudzik et al., 2000). Thus, it is evident that those cognitive abilities that are facilitated by 5-HT depletion correspond to cognitive processes underlying the function of the cerebral system in relaying in the cortico-striatal activity, and are tightly subject to DA modulation.

In previous experimental findings regarding both 5-HT and DA participation in cognitive processes, the pharmacological approach consisted of the application of neurotoxins that cerebrally deplete 5-HT or DA. The application of 5-HT reuptake inhibitors, which increase cerebral serotonergic activity, allows the entire 5-HT system to be manipulated using these global strategies. The availability of pharmacologically selective compounds for different subtypes of receptors has permitted the study of the different contributions of receptor subtypes on cognitive processing. However, the behavioural consequences after the increase or decrease in extracellular neurotransmitter levels caused by neurotoxins, or by the application of reuptake inhibitors, agonist and antagonists affecting global cerebral systems or

structures must be different to physiological changes in availability and release of neurotransmitter from afferent terminals into defined circuits and their effect on specific behavioural demands also changes.

The diverse physiological actions of DA are mediated through two receptor populations: D₁-like DA receptors and D₂-like DA receptors. D₁-type receptors include the subtypes D₁ and D₅, and are stimulators of adenylate cyclase, while D₂-type receptors include subtypes D₂, D₃ and D₄, which are inhibitors of adenylate cyclase (Kebabian et al., 1984).

DA receptors and cognition

It has been proposed that DA modulates spatial functions through the innervation arising in the SN and VTA to hippocampus (mainly into its ventral part), principally through D₂ DA receptors with minor participation of D₁ receptors (Wilkerson and Levin, 1999). However, evidence regarding the participation of D₁ and D₂ receptors in modulating spatial ability has not yet been well defined. For example, both place- and cue-learning were evaluated using a Morris maze after the administration of the D₁-like receptor antagonist SCH23390, and D₂-like receptor antagonist sulpiride, in rats. High doses of SCH23390 had an adverse effect on cue learning, whereas sulpiride in high doses impaired performance in the hidden platform test in the Morris maze (Stuchlik et al., 2007), apparently showing a dissociation of D₁- and D₂-like receptors in striatal- and hippocampal-dependent navigation tests (McDonald and White, 1993) in a manner where D₁ antagonism affected cue learning, but not place learning, whereas D₂ antagonism impaired place learning. However, in tests on place conditioning, the systemic administration of SKF 82958, a D₁ receptor agonist, induced place preference in rats (Abrahams et al., 1998). This is consistent with evidence that D₁ receptors are critical for long-term potentiation (LTP) and spatial learning in hippocampus, and are also related to the acquisition of new information through hippocampal LTP and long-term depression (LTD) (Lemon and Manahan-Vaughan, 2006). Moreover, in place

avoidance tasks that were developed to evaluate the organization of behaviour and spatial cognition (Cimadevilla et al., 2001; Vales and Struchlik, 2005), a recently established testing paradigm, the active allothetic place avoidance task (AAPA), which requires rats to actively avoid a room-frame-fixed shock sector in a continuously rotating arena (Stuchlik et al., 2004), was used to evaluate the participation of a D₁ receptor agonist and antagonist in the modulation of spatial cognition in rats. Application of the D₁ receptor antagonist SCH23390 (0.02 and 0.05 mg/kg i.p.) produced a deficit in the solution of a task, as evident from the higher number of entrances to the shocking (punishment) zone, as well as the shorter time that elapsed without entrance into the punishment zone. In contrast, application of lower doses of the D₁ receptor agonist (1*R*-*cis*)-1-(aminomethyl)-3,4-dihydro-3-tricyclo[3.3.1.1^{3,7}]dec-1-yl-[1H]-2-benzopyran-5,6-diol hydrochloride (A77636) produced an enhancement of efficiency, as measured by the small number of errors (Stuchlik and Vales, 2006). Similarly, other authors observed spatial navigation deficits after systemic administration of SCH23390 to rats solving a radial maze (Liao et al., 2002), supporting the more important role of D₁ receptors in spatial hippocampal-dependent tasks. However, when pretrained animals were evaluated in an AAPA task for reinforced retention under the influence of drugs after learning the test, no effect was observed for low doses of A77636 or SCH23390 in AAPA, but high doses of SCH23390 caused a motor deficit in the retention tests. From these observations, the investigators concluded that the effect observed on D₁ antagonist application was due to a non-spatial aspect of the task, and that this affected procedural aspects of the task (Stuchlik, 2007). This is very important, because only the procedural components of the task were affected in the tests once the spatial information was acquired, and thus, this effect may be principally dependent on striatal function, which as was previously mentioned, is preponderant in the organization of procedural learning (McDonald and White, 1993).

Thus, place navigation, working memory, conditioning and procedural learning are all processes organized principally or partially by a cerebral

system that is under the modulating influence of the DArgic system. Starting from this point of view, the effect of serotonergic modulation on these same cognitive abilities will be explored and related directly (when experimental evidence permits it) or indirectly from neurochemical data with the DArgic system in an integrative effort.

Serotonergic receptors and cognition

5-HT₁ receptor functions in 5-HT organized cerebral processes have been evaluated using 8-OH-DPAT, which initially considered the 5-HT_{1A} receptor agonist, and which has later been found to have a combined effect as an agonist of 5-HT_{1A/7} receptors (Eglen et al., 1997). The consequences of 5-HT_{1A} receptor activation were examined in different studies to evaluate spatial working memory. Deficiencies in working memory were observed after the systemic application of 8-OH-DPAT at doses of 0.2 and 0.5 mg/kg in pretrained rats. Briefly, the authors measured re-entry into baited or unbaited arms previously visited (working memory error) by rats that were trained to eat in four baited arms of a radial maze until they reached a criteria of three or more correct responses over five consecutive days. The number of working memory errors increased on agonist application, while no effect on the reference memory was observed (Carli et al., 1999; Isayama et al., 2001). The mediation of the effect of 5-HT_{1A} receptors was investigated by Egashira et al. (2006), who evaluated spatial working memory ability in an eight-arm radial maze after systemic 8-OH-DPAT administration or bilateral infusion into the dorsal hippocampus. The spatial working memory was impaired, and this impairment was blocked by the application of 5-HT_{1A} antagonists (NAN-190 and WAY-100635), while the administration of SB269970, a specific 5-HT₇ receptor antagonist, had no effect on the impairment caused by 8-OH-DPAT.

In a delayed non-matching to position task, which was used to evaluate working memory, Ruotsalainen et al. (1998) found that cerebral 5-HT depletion by 5,7-DHT application did not produce an effect, while 8-OH-DPAT application (100 µg/kg) reduced the probability of responding

to a sample lever without affect the choice accuracy. However, in a more recent study, [Fernández-Pérez et al. \(2005\)](#) who used a delayed non-matching to position task, to evaluate the effect of 8-OH-DPAT (0.3 mg/kg), the antagonist WAY-100635 and the 5-HT reuptake inhibitor fluoxetine, observed an effect on the accuracy of the response. 8-OH-DPAT and fluoxetine produced delay-dependent deficiencies in the response accuracy, and WAY-100635, which has no effect when applied alone, not only reversed the impairment caused by 8-OH-DPAT and fluoxetine, but improved the response accuracy in rats treated with fluoxetine. Thus, increased 5-HTergic activity through the administration of the 5-HT reuptake inhibitor fluoxetine or activation of the 5-HT_{1A} receptors, caused the deficiencies in the conditioned tests evaluating working memory (through response delay), and the effect was mediated through the 5-HT_{1A} receptors. Meneses and co-workers have extensively evaluated the manipulation of 5-HTergic receptors activity in an autoshaping test. Briefly, rats were trained to press a lever (presentation of the lever for a period of eight seconds), followed by administration of a food pellet if the animal pressed the lever (conditioned response). The trial was shortened, and the food pellet was delivered immediately, thus, the learning produced an increase in conditioned response that could be evaluated for STM (1.5 h after) and LTM (24 h after). Using this task, the authors evaluated the effect of post-training systemic administration of 8-OH-DPAT (dosages = 0.25 and 0.50 mg/kg), which produced impairments in STM and LTM ([Meneses, 2007](#); [Meneses et al., 2008](#)).

5-HT_{1A} receptors have been implicated in the regulation of spatial information processing. [Latgen et al. \(2005\)](#) evaluated the role of 5-HT_{1A} in spatial learning in rats using a water maze task. Pretraining administration of 8-OH-DPAT impaired water maze performance both at low doses (0.01 and 0.03 mg/kg) and high doses (0.1 and 1.0 mg/kg). The impairment was blocked by the 5-HT_{1A} receptor agonist NAD-299. An adverse effect of the activation of 5-HT_{1A} receptors has been observed in spatial information acquisition, whereas 5-HT_{1B} receptor activation,

through the application of the specific agonist CP 93129, preferentially impairs reference memory ([Buhot et al., 1995](#)). Thus, spatial working and reference memory are adversely modulated by 5-HT through 5-HT_{1A/B} receptors.

As described previously, antagonists to D₁ and D₂ receptors can interfere with hippocampal-spatial processing. Moreover, 5-HT_{1A} agonists administered systemically stimulate midbrain DArgic neurons, increasing DA release. Furthermore, 5-HT_{1A} receptors are located post-synaptically in corticolimbic areas innervated by DArgic cells including the PFC, amygdala and hippocampus ([Pompeiano et al., 1992](#)). Each area is related to spatial working memory processing. In the PFC, the application of 5-HT_{1A} receptors agonists in high doses decreases DA release ([Alex and Pehek, 2007](#)), in accordance with the effect of DA receptor antagonists causing deficiencies in spatial working tests. Moreover, [Fernández-Pérez et al. \(2005\)](#) have shown that in rats, the co-administration of fluoxetine and the 5-HT_{1A} receptor antagonist WAY100635 produces memory improvement in a delayed non-matching to position task. This provides evidence that the cognitive deficits caused by 5-HT_{1A} receptor agonism could be the result of their indirect actions on other neurotransmitter systems as DArgic system. However, no data exists that relates the 5-HT/DA interaction to deficits produced by 5-HT_{1A} receptor agonists on place learning. Rather, the effect on spatial function has been related to 5-HT/Ach interactive effects. However, a substrate for interaction between 5-HT and DA in modulation of spatial processing exists.

In associative learning, it has been reported that 8-OH-DPAT does not effect or retard acquisition ([Harvey, 1996](#)), while [Misane and Ögren \(2000\)](#) have consistently shown that 8-OH-DPAT impairs 24-h PA retention when systemically administered before training or retention, whereas no effect is seen after immediate post-training administration ([Ögren, 1985, 1986](#); [Misane et al., 1988](#)). However, these authors indicate that no apparent effect on DA systems by 8-OH-DPAT exists ([Misane and Ögren, 2000](#)). Other receptors participating in cognitive processes include the 5-HT_{2C} receptor that has principally been involved in the modulation

of associative learning. 5-HT_{2A/C} receptors are expressed post-synaptically in the neocortex, including the limbic cortex, the hippocampus, thalamus and basal ganglia. Several studies, including those on agonists of 5-HT_{2A/C} receptors 4-methyl-2,5-dimethoxyamphetamine (DOM), 3,4-methylenedioxamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) have shown an enhanced acquisition of conditioned responses. Moreover, the capability of 5-HT_{2A/C} receptor antagonists to retard learning and to block the enhancement produced by agonists indicates that these receptors are needed for normal associative learning (Harvey, 1996). 5-HT_{2A} receptors have been proposed as regulators of DA neuron activity through the regulation of cortico-tegmental projections, which in turn, regulate the activity of DArgic neurons (Pehek et al., 2006). Moreover, 5-HT_{2A} receptor agonists increase the activity in the nigrostriatal DArgic pathway, while their antagonism decreases the evoked release of DA (Alex and Pehek, 2007). Evidence exists that 5-HT_{2C} receptors inhibit DA release in striatum, AN and the PFC. In accordance with this, Boulougouris et al. observed an effect of the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SB242084 in a serial spatial reversal learning task. Any of these antagonists altered the discrimination or retention of the response previously acquired, but only the 5-HT_{2A} antagonist impaired reversal learning, manifested in an increase in perseverative responses, whereas the 5-HT_{2C} receptor antagonist improved reversal learning, as measured by a decrease in the number of trials required to attain the criteria, and by a reduction in perseverative responses (Boulougouris et al., 2008). Thus, the activity of 5-HT through these receptors could account, at least partially, for the detrimental effect of 5-HT depletion on reversal learning. Because of the participation of the PFC in reversal learning, the effect of this compound could be occurring at this level under prominent DArgic control, for which DA/5-HT interactions could be occurring, since the 5-HT_{1A} antagonists could mediate their effect through a reduction in DA release, whereas the 5-HT_{2C} antagonists block the inhibitory effect of 5-HT through these receptors on release of DA.

PA is affected by manipulation of 5-HT₄ receptors, the highest density of which, occur in the hippocampus, frontal cortex and amygdala, which are all regions related to cognitive processes (Eglen et al., 1995). The application of the partial 5-HT₄ receptor agonist SL65.0155 (1 mg/kg/day), 7 days before a test of PA in mice showed an enhancement of the PA response, as evidenced by an increase in the latency of re-entry to the apparatus compared to vehicle-treated animals, after 1 day of the training trial. Moreover, this agonist is able to reverse amnesic effects caused by the administration of galanin (Micale et al., 2006), a compound that, when administrated in the lateral ventricles, inhibits acetylcholine release and produces deficits in learning and memory (Kinney et al., 2003). In PA tests in mice, the application of 5-HT₄ receptor antagonists immediately after training produced an amnesic effect, which was prevented by 5-HT₄ receptor agonists (Galeotti et al., 1998). Moreover, 5-HT₄ receptor partial agonists facilitated both STM and LTM (5.0 and 10.0 mg/kg, respectively) in an autoshaping test (Meneses, 2007). Biochemical evidence indicates that 5-HT₄ receptor stimulation is able to increase the release of striatal DA in vivo. Moreover, other evidence indicates that this receptor modulates impulse-mediated DA release, whereas it does not affect tonic release. The modulatory effect appears to occur through the action of 5-HT₄ receptors located on SN neurons as well as by direct action on striatal elements (Alex and Pehek, 2007). Thus, it is possible to propose that the facilitating effect observed after stimulation of 5-HT₄ receptors can be mediated by an increase in DA activity. However, despite the indirect evidence, it has not been evaluated whether changes produced by 5-HT₄ receptor stimulation on PA are mediated by changes in DA.

5-HT₃ receptors are related to cognition, although the principal effects observed regarding their participation concern the amelioration of deficits caused by acetylcholine depletion (Barnes et al., 1990). However, an effect of these receptors on learning and memory has been reported. Post-training intraperitoneal administration of the agonist mCPBG impaired retention in auto-shaping tests, whereas the antagonists tropisetron

and ondansetron improved retention (Hong and Meneses, 1996; Meneses, 2007). Granisetron, another 5-HT₃ receptor antagonist, induced deficiencies in spatial learning in a Morris water maze when it was infused intra-hippocampally 20 min before daily training without having any effect on visual discrimination (Naghdi and Harooni, 2005). Previously Staübli and Xu (1995) observed that 5-HT₃ receptor antagonists enhanced hippocampal learning and modulated hippocampal plasticity (LTP).

Moreover, the over-expression of 5-HT₃ receptors in mice produced enhanced contextual conditioning, without having an effect on cued conditioning and latent inhibition enhancement, as well as inducing a heightened exploratory behaviour and a decrease in anxiety, as measured in an elevated-plus maze (Harrell and Allan, 2003). 5-HT₃ receptors are primarily localized pre-synaptically as heteroreceptors (MacDermott et al., 1999). Their localization allows for the modulation of the release of neurotransmitters, such as DA (Campbell et al., 1996; Allan et al., 2001). It has been shown that in striatum, these receptors are present in potentially DArgic nerve terminals (Nayak et al., 2000), where they can increase evoked neurotransmitter release (Rondé and Nichols, 1998; Alex and Pehek, 2007). PFC application of 5-HT₃ receptor agonists induces an increase in DA release and the antagonists produce a decrease. However, no evidence exists relating the effect of 5-HT₃ receptor agonism on cognition and its effect on the DA system, although it has been implicated in motor control through its interaction with nigrostriatal DA neurons by its control of depolarization-dependent exocytosis only when central DA and 5-HT tone are concomitantly increased (Porrás et al., 2003).

Earlier studies have indicated that 5-HT acting through 5-HT₆ and 5-HT₇ receptors had no effect on improving memory formation or reverting amnesia. 5-HT₆ and 5-HT₇ receptors are distributed in areas involved in memory formation as striatum, AN, hippocampus and frontal cortex (Gerard et al., 1997). Using the 5-HT₆ receptor antagonist RO4368554, Schreiber et al. (2007) tested scopolamine-impaired and unimpaired male

rats in several cognitive tests. They found that RO4368554 reversed the effects of scopolamine in novel object discrimination and PA, whereas it enhanced the performance in unimpaired animals in object discrimination after an interval of 4 h. No effects were observed on Morris water maze performance by the administration of RO4368554 to unimpaired animals. The effects of post-training systemic application of the 5-HT₆ receptor agonist EMD in an associative autoshaping task (1–10 mg/kg) were tested both in STM and in LTM. The agonist at a dosage of 5.0 mg/kg impaired both short- and long-term memory. The 5-HT₇ receptor agonist AS19 was applied using the same procedure and dosages, and (2S)-(+)-5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino) tetralin (AS19) significantly impaired STM (Meneses, 2007; Meneses et al., 2008).

Gasbairri et al. (2008) evaluated the effect of the 5-HT₇ receptor antagonist SB-269970 on radial arm maze performance using a procedure involving two phases to evaluate working memory during an acquisition phase and reference memory during a phase test. In this test, the antagonist improved memory in the test phase by affecting the reference memory, but had no effect on working memory. Whereas much data supports the 5-HT₆ receptor modulation of cognitive ability through actions on cholinergic neurons (Mitchell and Neumaier, 2005), less evidence exists regarding 5-HT₆/DA and 5-HT₇/DA interaction.

Thus, 5-HT, through its diversity of receptors, regulates different cognitive abilities, producing different effects that depend on the nature of the information processed (e.g., egocentric vs. allocentric information), and on the type of processing of the information (e.g., stimulus–response vs. stimulus–stimulus associations and STM vs. working memory). In the processing of information directed by cerebral regions under strong DArgic modulation, such as the striatum and the PFC, 5-HT antagonism or depletion appears to induce a facilitating effect, whereas 5-HT agonism appears to have a detrimental effect. Thus, a potential interactive effect of 5-HT/DA could be occurring in the organization of such abilities in these cerebral structures (Table 2).

Table 2. Effect of 5-HT receptors agonist and antagonist administration on learning and memory tasks in rat

Receptor	Compound/ admon. via	Spatial function	STM/LTM	Working memory	Avoidance	Conditioning
5-HT1A/7 Ago	8-OHDPAT/ip	WM–		DNMPT–	AP+ Low dose AP– High dose AP =, –	
5-HT1A Ant	8-OHDPAT/IHB	RAM–		RAM–		
5-HT1B Ago	WAY-100635/ip	RAM–	DNMPT+ /	DNMPT+ /		
5-HT2 A Ago	CP 93129/ip			RAM =		IC+, —
5-HT2A Ant	DOM, MDA, MDMA/ip					
5-HT2C Ant	M100907/ip					RL-IC —
5-HT3 Ago	SB242084/ip					RL-IC +
5-HT3 Ant	MCPBG		AST–			
5-HT4 Ago	Ondansetron, Tropisetron	WM +	AST +			
5-HT4 Ant	SL65.0155/ip				PA +	
	SDZ 205557, GR125487				PA–	
5-HT6 Ago	EMD/ip		AST–/–			
5-HT6 Ant	RO4368554/ip	WMPL =			PA +	
5-HT7 Ago	AS19/ip		AST–/ =			
5-HT7 Ant	SB 269970	RAM +		RAM =		

Symbols: =, no changes; +, facilitation effect; –, detrimental effect. Abbreviations: BM, Biel's maze; WM, water maze; RAM, radial arm maze; ST, Stone's maze; TM, T maze; YM, Y maze; DeA, delayed alternation; DNMPT, delayed non-matching to position tests; S, simple conditioning; Go, go tests; no-go, no-go tests; PA, passive avoidance; AA, active avoidance; WMCL, water maze cue learning; WMEL, water maze egocentric learning, IHB, intra hippocampal bilateral; AST, autoshaping test; RL-IC, reversal learning in instrumental conditioning task. Chemical compounds, see abbreviations list.

Striatum and cognitive processes

Striatal mediated cognitive processes and DA

The striatum (caudate–putamen) part of the basal ganglia is a group of subcortical nuclei that also includes the subthalamic nucleus, globus pallidus and SN (Wilson, 1998). These nuclei participate in voluntary movement regulation, frequently called motor control, and are affected by diseases such as Parkinson's and Huntington's diseases: both diseases cause dementia because cognitive functions sustained by the basal ganglia are altered with the degeneration of these nuclei (Packard and Knowlton, 2002).

The striatum constitutes the incoming pathway for cortical inputs (Packard and Knowlton, 2002), and has been associated with several experimental studies on procedural learning, sequential motor learning (implicit sequential motor information is a form of procedural learning), conditioning and

the establishment of stimulus–response associations, as well as egocentric learning (McDonald and White, 1994, 1996). These cognitive processes are directed through its interaction with the cerebral cortex via the arrival of aforementioned afferent information (Wilson, 1998; Packard and Knowlton, 2002). Temporal changes during a rewarded instrumental conditioning task, in which rats were trained to press a lever after a tone to obtain a food reward, were measured by Nakazato (2005). The reaction time for pressing the lever was measured as an index of learning, and the concentration of DA in the ventral anterior striatum was measured every week for a period of 5 months. The concentration of DA began to increase just after the cue presentation, and reached a peak near the time of pressing the lever, returning to the base level 1–2 s after pressing the lever. These changes were observed during the 5 months of training. The peak in DA after the cue presentation was higher when the task was not yet

perfected, and subsided towards the end of the training period. This indicates that increased DA release is required for instrumental conditioning acquisition and stops when the task is learned.

Stimulus–response habit formation was evaluated after bilateral intrastriatal administration of 6-OH-DA to rats. Whereas the control animals required six training sessions to reach a criterion, 6-OH-DA-injected rats did not reach the criteria until the twelfth session, i.e., the lesioned animals were slower to acquire the instrumental actions, but after over-training, these animals performed similar to control animals. Moreover, the control animals did not show reward devaluation by specific satiety, which is indicative of habit formation, while the lesioned rats did show reward devaluation, which indicates that their stimulus–response was directed by goal expectancy, that is, no habit formation occurred (Faure et al., 2005). After unilateral nigrostriatal lesions, Hudzik et al. (2000) observed that rats were deficient in acquiring an operant task when pretrained animals were evaluated in a test requiring the achievement of response–duration differentiation, the unilateral lesions produced marked deficiencies too. There is also evidence that the dorsal striatum (or caudate–putamen) is involved in aversive conditioning. Lesions of this structure produce deficits in active avoidance (Kirkby and Polgar, 1974; Winocur, 1974) and PA (Winocur, 1974; Prado-Alcala et al., 1975).

In aversive conditioning, a dissociation of functions for hippocampal and striatal processes has been observed. In Pavlovian aversive conditioning effected by the presentation of three-paired tones and foot shocks, application of D-amphetamine immediately post-training in either hippocampus or dorsal striatum causes a reduction in conditioned freezing measured 24 h later. However, this is only in response to contextual information by the hippocampal administration in rats and both for contextual and tone response for striatal infused rats. Thus, the dorsal striatum is involved in aversive conditioning to both contextual and discrete conditioned stimuli (White and Salinas, 2003). Dunnett and White (2006) observed an impairment in choice accuracy in an operant test of delayed alternation after bilateral

striatal lesions in rats, in both tests without a delayed response and in tests with a delayed response. This indicates an effect on the operant aspects of the task by the lesions more than alterations in working memory processes. Accordingly, the effect of unilateral or bilateral DA depletion, either in dorsal or ventral striatum, on the preparation and execution of a delayed response task in rats was evaluated by Florio et al. (1999). They observed that the dorsal striatum is related to stimulus–response learning because the animals lesioned in this area showed a lack of conditioned response, whereas the ventral striatum is related to temporal expectation and resulted in premature or omission responses. Specifically, the dorsolateral region of the striatum has been related to procedural tasks and habit learning (Featherstone and McDonald, 2004; Yin et al., 2004).

Thus, with regard to striatum-dependent cognitive abilities, a role for DA in consolidation processes is well-documented (Castellano et al., 1991; Packard and White, 1991; Packard and McGaugh, 1994). Direct DA receptor agonist administration increases the retention of information in several tasks, including several versions of the radial arm and Morris water maze (Castellano et al., 1991; Packard and White, 1991; Packard and McGaugh, 1994). These effects are thought to be mediated by both D₁ and D₂ receptors in mice (Castellano et al., 1991), while in rats, D₂ receptors have been more strongly implicated in early consolidation processes, whereas D₁ receptors may play a more important role in later stages of learning (White et al., 1993). Moreover, it has been proposed that the concerted participation of D₁ and D₂ receptors is required in organizing the striatal-dependent cognitive ability (Wolterink et al., 1993; Watanabe and Kimura, 1998), and the co-activation of D₁ and D₂ receptors is required for LTD in the striatum (Calabresi et al., 1992), a mechanism proposed as underlying the memory processes (Fino et al., 2005).

In rats, it is generally observed that D₁-like receptor agonists impair, whereas D₂-like receptor agonists enhance, responses in conditioning tests (Abrahams et al., 1998; Sutton et al., 2001). Shapovalova and Kamkina (2008) evaluated the effect of a bilateral blockade of DA receptors on

cognitive functions. They compared the systemic and intra-striatal application of the D₁ receptor antagonist SCH23390 on the acquisition of a discriminative conditioned active avoidance reflex in a T maze, as well as the effect on motor activity in the open field. The antagonist caused a marked reduction in the number of conditioned responses (0.025 mg/kg) and reduction in motor activity in the open field. Striatal bilateral D₁ receptor blockade did not have an effect on the conditioning, but affected the motor activity, causing a profound inhibition. Similar adverse effects on conditioning were observed after a bilateral infusion of the D₂ receptor antagonist raclopride without any changes in motor activity. Thus, the effects observed in conditional learning after systemic application of D₁ receptor agonist appear to be indirectly mediated, and occur through striatal D₂ receptors (Shapovalova and Kamkina, 2008).

Rats trained to release a lever in response to a visual cue within a reaction time limit were systemically administered with D₁ (1-[(2-bromo-4,5-dimethoxyphenyl) methyl]-L, 2,3,4-tetrahydro-6-methoxy-2-methyl-7-isoquinolinol (A69024)), D₂ (eticlopride) and D₃ (nafadotride) receptor antagonists. The D₁ receptor antagonist had no effect, whereas the D₃ receptor antagonist produced a mild effect on performance in high doses (1 mg/kg s.c.) consisting of an increase of delayed responses. The D₂ receptor antagonist produced profound deficits in performance in a dose-dependent decrease in number of correct responses (0.005, 0.01 and 0.02 mg/kg s.c.), caused by an increase in the number of delayed responses and an increase in reaction time (Smith et al., 2000). Lesions to the prefrontal medial cortex with 6-OH-DA, reducing DA levels in the PFC, did not alter the acquisition of fear conditioning. However, lesioned rats showed a delayed extinction of the conditioned response without an alteration of the initial acquisition (Morrow et al., 1999). This effect could be mediated by the D₂ receptors, because a facilitation of extinction in a conditioned fear test was observed by applying the D₂-receptor antagonist sulpiride, after a conditioning session pairing a tone with foot shock and pre-submission of the animals to extinction training (consisting of the

repeated CS presentations alone to generate extinction in mice). The extinction was measured during the extinction training and 24 h after the training in a free-drug condition; sulpiride treatment before the extinction training facilitated extinction memory 24 h later, and quinpirole (D₂ receptor agonist) partially blocked the extinction (Ponnusamy et al., 2005). The D₁ antagonist SCH 23390 (0.1–1 mg/kg) administered before the conditioning training inhibited the acquisition of conditioning freezing in tests carried out 24 h later. When it was administered after the foot-shock training, SCH 23390 did not affect the conditioned freezing (Inoue et al., 2000). Thus, both D₁ and D₂ receptors contribute to aversive conditioning. Although opposing effects in the electrophysiology of striatal spiny neurons mediated by D₁ and D₂ receptors have been reported, it has been proposed that a tonic D₂ receptor-mediated inhibition of synaptic efficacy may be important in suppressing striatal output when cortical activity is relatively low, and when the D₁ receptor activation is capable of depolarizing the membrane further, facilitating spike discharges. Thus, by controlling the excitability of striatal neurons via distinct effects on membrane activity and afferent drive, the DArgic system exerts a true modulatory influence over information processing in the striatum (West and Grace, 2002). Moreover, co-operative relationships between D₁ and D₂ receptors on striatum-dependent cognitive processes have also been shown. D₁ and D₂ receptors act simultaneously in mediating the cellular effects of DA regulating DA and glutamate release, and integrating DA with other neurochemical inputs to the striatum (Kiyatkin and Rebec, 1999). 5-HT could be one of the neurochemical inputs with a large repercussion on striatal DA-dependent cognitive processes.

Striatal cognitive processes and 5-HT

Clinical reports indicate that 5-HT function deteriorates in cerebral areas related to cognitive processing in patients with Alzheimer's disease (Wenk et al., 1987), and PD patients show alterations of 5-HTergic neurotransmission besides a decline in DArgic (Graybiel, 1990).

Intrastriatal (Yu and Liao, 2000) or systemic methamphetamine exposure causes monoamine depletion, and impairs sequential motor learning in a radial arm maze (Chapman et al., 2001). These motor learning deficiencies are associated with depletion of monoamines in striatum. A negative correlation between DA depletion in medial striatum and an increase in the number of direct movements (an index of sequential motor learning) was observed, as well as a correlation between the content of 5-HT in the lateral striatum and the number of direct movements (in both cases, the depletion of neurotransmitter was associated with a smaller increase in the number of direct movements) (Daberkow et al., 2005).

Other experimental work indicates that 5-HT participates in modulation of memory and striatal-dependent learning processes in a complex manner. Early work has shown that an increase in 5-HT activity attenuates conditioned reward response, whereas reducing cerebral 5-HT activity selectively enhances conditioned responses (Fletcher et al., 1999). In addition, Prado-Alcalá et al. (2003a, b) reported that intrastriatal administration of the 5-HT releasing drug PCA, or application of 5-HT, produces deficiencies in inhibitory avoidance tests. When 5-HT or PCA was striatally administered pretraining (30, 15 or 5 min before training) and the retention measured 24 h later, an inversely related time-dependent deficit was found (Solana-Figueroa et al., 2002). Both tests were strongly dependent on DArgic function, as previously stated. Serotonergic DRN neurons project to basal ganglia predominantly in striatum, and send axonic collaterals to SN (Ferré et al., 1994; Gervais and Rouillard, 2000), and either stimulation of the DRN (Hervé et al., 1979; De Simoni et al., 1987) or the administration of 5-HT induce changes in striatal DA release both in vivo and in vitro (De Belleruche and Bradford, 1980; Blandina et al., 1989). It has been suggested that serotonergic modulation is more evident during DA system activation (Palfreyman et al., 1993), which is known to occur during the establishment of conditioning (Rebec et al., 1997; Robinson et al., 2001). The effect of 5-HT or PCA directly infused into the striatum could involve a direct effect on DA function, since, 5-HT exerts a

presynaptic inhibitory action on cholinergic and DArgic terminals through 5-HT₂ receptors, and an inhibitory action on striatal cells through 5-HT₁ receptors. DA and ACh coactivation is required by the plastic changes that subserve striatal-mediated learning (Suzuki et al., 2001). According to this hypothesis, the post-trial intrastriatal infusion of the 5-HT₂ receptor antagonist ketanserine produces an amnesic estate because of a lack of inhibition of DA release in striatum that in turn, could induce a reduction in cholinergic release by inhibition through D1 receptor activation (Ramírez et al., 1997).

Interaction between 5-HT/DA has been observed in tests on serial reaction. Profound 5-HT central depletion by ICV application of 5,7-DHT was established in rats trained to detect and locate brief visual stimuli randomly presented in one of five spatial positions. After the establishment of a performance criterion fixed at more than 80% of correct responses were attained, 5-HT was depleted, and later, the performance of 5-HT-depleted rats was as accurate as control animals, but a reduction in the proportion of omitted responses, and an increase in premature responses, indicated an increase in the impulsivity of these animals. On the other hand, an increase in the proportion of premature responses obtained after systemically administered D-amphetamine was abolished by the 5-HT depletion, and reduced the decrease in correct responses induced by the application of the D₂ receptor antagonist (–)-sulpiride. However, systemic administration of the D₁ receptor antagonist SCH23390 blocked the impulsiveness increased by the 5-HT depletion. This is relevant, because it implies that impulsiveness generated by 5-HT depletion is mediated by the activation of D₁ receptors under conditions of low 5-HT concentration, and because the accuracy of responses was not affected by the decrease in 5-HT, but was able to attenuate the deficiencies mediated by D₂ receptor antagonist application (Harrison et al., 1997). Both findings indicate a relationship between the modulation of impulsiveness and attentional performance by 5-HT/DA interactive mechanisms. It is important to note that experimental prefrontal 5-HT depletion, which produces a reversal of learning deficiencies,

could be related to the increase in impulsiveness, and the possibility exists that a similar mediation of D₁ receptors on impulsiveness after 5-HT depletion occurs in the PFC. However, this possible 5-HT/DA interaction has not been evaluated.

Another striatal-dependent cognitive process is egocentric learning, which has been related to the neural memory system, including the striatum body (caudate–putamen) (McDonald and White, 1994, 1996) based on the evidence that lesions of the dorsal striatum disrupt the capability of rats to display egocentric responses (Brasted et al., 1997). Thus, rats with a caudate–putamen lesion are unable to use egocentric strategies (DeCoteau and Kesner, 2000). Striatal inactivation by lidocaine application induces an inability of the animals to use egocentric strategies without affecting the use of spatial allocentric information (Packard and McGaugh, 1996). Included among the behavioural tests in which animals showed a better performance after cerebral 5-HT depletion are those with strong egocentric components, that is, tests that include the learning of a sequential series of left–right turns. As previously mentioned, cerebral 5-HT depletion produces spatial egocentric learning facilitation (Olvera-Cortés et al., 2001). The effect was apparently striatum-mediated, because Anguiano-Rodríguez et al. (2007) evaluated the effect of striatal 5-HT depletion by intrastriatal application of 5,7-DHT on egocentric learning, and observed a facilitation of performance in rats. This task is difficult, because the animals were only permitted the use of proprioceptive information. Intact rats were unable to learn this task in the ten trials constituting the test. Striatal-5-HT depleted rats successfully learnt the task in the ten trials. The facilitation was blocked by intrastriatal infusion of mixed D₁ and D₂ receptor antagonists (sulpiride and SCH23390), and was reinstated once the blockade of DA receptors was removed. This work implies that the DArgic system sustains the facilitating effect of 5-HT striatal-depletion on egocentric learning, but the precise mechanism remains unknown. In addition to the several mechanisms mentioned above that could underlie 5-HT/DA interaction in the modulation of cognitive process, it has been observed that free striatal

serotonergic terminals can modulate striatal activity (Soghomonian et al., 1989). An increase in DA and NA release in the frontal cortex, accumbens, and striatum has been reported to occur after administration of a 5HT_{2C} receptor antagonist (Gobert et al., 2000). Moreover, 5-HT modulates acetylcholine, γ -aminobutyric acid (GABA), DA, and 5-HT release through 5HT₄ receptors (Barnes and Sharp, 1999). Thus, 5-HT modulates striatal DA release through 5HT₄ receptor activation, whereas inhibition occurs when 5-HT_{2C} receptors are stimulated (Alex et al., 2005). A growing body of evidence has highlighted the potential of central 5-HT_{2C} receptors for an improved treatment of neuropsychiatric disorders related to DA (Wood et al., 2001). 5-HT_{2C} receptors are expressed along striatal and mesocortico-limbic DArgic pathways, and exert phasic and tonic inhibitory controls of both basal DA neuronal firing and basal DA release in the AN, striatum and frontal cortex (Di Giovanni et al., 1999; 2000; Gobert et al., 2000). 5-HT_{2C} receptors potentiate the increase in DA release induced by drugs that stimulate DA neuronal firing, such as morphine (Hutson et al., 2000). Thus, it has been proposed that 5-HT_{2C} receptors selectively modulate impulse-dependent release of DA in the accumbens and striatum, but only when DA release is associated with increased firing of DArgic neurons (Willins and Meltzer, 1998; Lucas et al., 2001). Through 5-HT₆ receptors, 5-HT affects the nigrostriatal function through acetylcholine release regulation (Bourson et al., 1998), blockade of 5-HT₆ receptors causes an increase in acetylcholine release, which in turn causes that the release of glutamate increases, along with increases in the performance in memory tasks (Sleight et al., 1999; Roth et al., 1994).

The relationship between 5-HT₆ receptor activity and DArgic function has been delineated, but there is confusion about the extent to which this interaction occurs (Mitchell and Neumaier, 2005). Apparently, 5-HT₆ receptor blockade potentiates DA transmission by stimulatory drugs, such as amphetamine (Frantz et al., 2002). In the striatum, the role of 5-HT₆ receptors on DA was evaluated in presence and absence of the DA transporter inhibitor/releaser amphetamine. Subcutaneous

administration of amphetamine induces an increase in extracellular DA, which is increased by SB-271046 (5-HT₆ receptor antagonist), and generates an increase in 5-HT. Local infusion of amphetamine into the striatum induces an increase in DA, but no effect was observed after coadministration of a 5-HT₆ receptor antagonist. Thus, 5-HT₆ receptors can modulate the striatal DA and 5-HT systems when DA neurotransmission is enhanced (Dawson et al., 2003).

Thus, many of the actions of 5-HT can be related with its modulation of the DA neurotransmitter system, especially those cognitive processes that engage corticostriatal functioning. However, few studies that relate 5-HT and DA exist, despite the extensive biochemical evidence.

Prefrontal cortex and cognitive processes

The PFC constitutes a higher level in the cortical hierarchy involved in the representation and execution of actions. In addition to cognitive control, the PFC plays a crucial role in behavioural control and influence (Fuster, 1997; 2001; Miller and Cohen, 2001). The key cellular elements in the direction of these functions are the pyramidal neurons, and the basic process in which the PFC participates is working memory: an essential process for human cognition (Goldman-Rakic, 1995).

The PFC constitutes the more rostral region of the frontal lobe, with anatomical landmarks imprecise in diverse mammal species. However, in all species, the PFC possesses a reciprocal connectivity with the mediodorsal thalamic nucleus (Groenewegen and Uylings, 2000; Fuster, 2001). In primates, the PFC comprises Brodmann's areas 8–13, 24, 32, 46 and 47 (Brodmann, 1909). The PFC collectively consists of an interconnected network of subregions that send and receive projections from virtually all cortical sensory and motor systems, as well as a number of subcortical structures. Findings from human, non-human primate and rodent species suggest that specific aspects of cognitive processing are differentially weighted across distinct subregions of the PFC. The lateral and mid-dorsal PFC are thought to be closely associated with sensory

processing, and these regions receive auditory, visual, and somatosensory information from temporal, occipital, and parietal cortices (Goldman-Rakic and Schwartz, 1982; Barbas and Pandya, 1989). Thus, the dorsolateral region integrates information involved in the temporal organization of behaviour, working memory, language and reasoning (Vertes, 2004). The medial PFC, along with orbital regions, shares connections with limbic structures critical for memory and the processing of internal states, such as motivation and affects (Amaral and Price, 1984; Barbas and De Olmos, 1990). This region is also thought to be important for the process of behavioural inhibition (Fuster, 1980; Goldman-Rakic, 1987). Neuroanatomical studies divide the PFC of rats into three principal regions: lateral, orbital and medial. The latter region is also sub-divided into three zones (in dorso-ventral order): cingulate anterior (CG1), prelimbic (PL) and infralimbic (IL). Although the functional subdivision of these regions in rats is not well defined, recent studies infer from the projection pattern of the PL and PI zones, that the first region could be related to limbic-cognitive functions (homologues to the PFC of primates) and the second region participates in the control of visceral-autonomic activities (homologues to the orbital PFC of primates) (Vertes, 2004).

Prefrontal cortex and DA

It has long been recognized that DA in the PFC is critical in regulating cognitive processes, such as working memory, behavioural flexibility and decision making. These functions are disrupted in patients suffering with schizophrenia. Moreover, it is now understood that some optimal level of extracellular DA must be maintained within the cortex to sustain normal executive functioning, in that too much, or too little, cortical DA produces cognitive dysfunction (Goldman-Rakic et al., 2000; Winterer and Weinberger, 2004). Hypotheses concerning the role of DA in cognitive functions have focused on its ability to modulate executive functions (Sawaguchi and Goldman-Rakic, 1991; Zahrt et al., 1997; Roitman et al., 2004). During the performance of a delayed alternation task, a measure of working memory,

monkeys displayed an increase in prefrontal DA release (Matsuda et al., 2001). In addition, both overstimulation and inhibition of the prefrontal DA system have been shown to decrease working memory performance (Sawaguchi and Goldman-Rakic, 1991; Aultman and Moghaddam, 2001; Abi-Dargham et al., 2002; Kellendonk et al., 2006). The capability of both insufficient and excess DA to decrease performance in tasks of cognitive functioning further emphasizes the importance of a proper balance of DArgic activity. The ability of DArgic agents to influence performance of specific cognitive tasks, while leaving performance of others intact, provides further support for a tightly regulated and specialized role for DA in prefrontal cortical function (Briand et al., 2007).

The mesocortical system originates in the VTA, terminating in the frontal cortex, forming synapses with cortical pyramidal glutamatergic neurons and non-pyramidal GABAergic interneurons (Fluxe et al., 1974). Furthermore, the VTA receives reciprocal input from the PFC (Sesack and Pickel, 1992). Afferents from the PFC innervate the mesoaccumbens GABAergic, but not the DArgic cells, as well as mesofrontal DArgic, but not the GABAergic neurons (Sesack and Carr, 2002). This specificity of the PFC innervations creates a one-to-one relationship with prefrontal efferents synapsing onto VTA DArgic cells that form reciprocal prefrontal connections. Possibly, this input is important for facilitating learning through the influence of prediction errors (Schultz, 1997; Schultz et al., 1997).

DArgic transmission in the PFC is mediated by two DA receptor subtypes: D₁ and D₂ receptors. Although both receptor subtypes are present in the PFC, they display only a partially overlapping distribution. D₂ receptor expression is considerably less dense than that of D₁ receptors. D₂ receptors are found almost exclusively in Layer V, while D₁ receptors are most densely distributed in the superficial layers (Layers I–III), although they can be found in all layers (Goldman-Rakic et al., 1990). The differential distribution of D₁ and D₂ receptors is important due to their different second messenger cascades. D₁ receptors are coupled to stimulatory G proteins, while D₂ receptors are coupled to inhibitory G proteins (Kebabian et al., 1984). Along with

these differences in signalling mechanisms, D₁ and D₂ receptors exhibit differences in binding affinity, with D₂ receptors responding to much lower levels of DA than D₁ receptors do (Grace, 2000).

The VTA DArgic neurons discharge in both tonic and phasic fashions, and these firing patterns result in tonic and phasic release of DA in the PFC (Stoof and Kebabian, 1981; Grace, 1991). Tonic DA release is activated by sustained increases in DA neuronal firing, or presynaptic stimulation of DA terminals by glutamate. In contrast, phasic DA release results from spike-dependent mechanisms, and occurs in response to behaviourally relevant stimuli (Finlay et al., 1995; Rebec et al., 1997).

Several studies have demonstrated the strikingly prolonged effect of DA release/application on PFC activity. For example, in vitro electrophysiological studies have shown that bath application of DA for 2–5 min produces modulations in current PFC interneurons (Gorelova et al., 2002) and pyramidal neurons (Gorelova and Yang, 2000) that lasts for tens of minutes, or until the recording is no longer viable (Seamans and Yang, 2004). Interestingly, these protracted increases in cortical current are generally D₁ receptor, and not D₂ receptor, dependent (Gorelova et al., 2002). This bidirectional characteristic of DA is particularly apparent regarding its modulation of key synaptic currents that regulate cortical activity, such as GABA and *N*-methyl-D-aspartate (NMDA) currents (Seamans et al., 2001b; Durstewitz and Seamans, 2002). For example, DA modulation of GABAergic inhibitory post-synaptic currents (IPSCs) in deep layer pyramidal neurons initially produce a D₂-mediated reduction in IPSC amplitude, followed by a longer lasting D₁-mediated increase in evoked IPSCs (Seamans et al., 2001a; González-Burgos et al., 2005).

The initial D₂-mediated effect is believed to occur via a novel signalling pathway involving inositol phosphate (IP₃) receptors and increased intracellular Ca²⁺ (Trantham-Davidson et al., 2004), whereas the D₁-mediated effect occurs via a cAMP/PKA-dependent cascade in interneurons (Gorelova et al., 2002; Trantham-Davidson et al., 2008). D₂-receptor activation also suppresses NMDA currents (Zheng et al., 1999), and Tseng and

O'Donnell (2004) reported that this suppression was blocked by the GABA antagonists bicuculline and picrotoxin, suggesting that the inhibitory action of D₂ receptors on NMDA-induced responses in the PFC is mediated by GABAergic interneurons. In contrast, activation of post-synaptic D₁/D₅ receptors increases the NMDA component of excitatory post-synaptic (EPSCs) in the PFC (Seamans et al., 2001b; Chen et al., 2004).

Zheng et al. (1999) demonstrated that DA's effect on cortical NMDA currents was concentration dependent. Specifically, at lower concentrations (10 μ M), DA enhanced NMDA currents in the PFC (via D₁-like receptors), whereas at high concentrations (100 μ M) DA decreased the current (via D₂-like receptors). According to this, it is believed that transitions between D₁ and D₂ receptor activation help stabilize cortical representations in the working memory (Seamans and Yang, 2004; Winterer and Weinberger, 2004; Durstewitz and Seamans, 2006). In all previous reports, it has been proposed that in the pathological PFC, abnormal cortical D₁/D₂ activation ratios, along with altered GABA and glutamate transmission, interfere with this process. In particular, D₂-receptor stimulation of PFC pyramidal cells suppresses GABAergic and NMDA currents sufficiently to produce a permissive state in the cortex; putatively allowing multiple representations to be held in cortical networks simultaneously (Di Pietro and Seamans, 2007). This initial bias in the activation of D₂ receptors is important in situations requiring response flexibility and open-ended problem solving, where many options for action must be held in the memory and compared (Seamans and Yang, 2004).

Prefrontal cortex and 5-HT

Serotonergic projections to the cortex arise primarily from the DRN and MRN (O'Hearn and Molliver, 1984). The DRN consists primarily of ipsilateral projections to the frontal cortex, while the MRN projects bilaterally to frontal, parietal and occipital cortices (O'Hearn and Molliver, 1984; Jacobs and Azmitia, 1992).

While it is clear that the dorsal raphe send ascending projections to the PFC, it has only

recently been determined that it also receives reciprocal connections from the PFC (Peyron et al., 1998; Celada et al., 2001).

Although many of the different receptor subtypes are located in the PFC, their specific neuronal locations (post-synaptically vs. somatodendritically and pyramidal cells vs. GABA interneurons) may allow for highly specific serotonergic effects on post-synaptic targets. The 5-HT_{2A} receptor is the predominant 5-HT receptor found in the cortex, where it is located on all cortical pyramidal cells, as well as parvalbumin- and calbindin-containing GABAergic interneurons. While the action at 5-HT_{2A} receptors on GABAergic neurons is known to be involved in perisomatic inhibition of pyramidal cells, on pyramidal cells this receptor subtype is located post-synaptically, and its activation increases the excitability of the PFC neurons (Harvey, 1996; Buhot, 1997; Jakab and Goldman-Rakic, 2000). The 5-HT_{1A} receptor is also found on the majority of pyramidal neurons and on more than 25% of the GABAergic interneurons (Buhot, 1997; Gu, 2002). These receptors are located somatodendritically and are generally thought to decrease neuronal excitability (Buhot, 1997).

Research over the last decade supports the hypothesis that the DRN 5-HT system plays a specific role in prefrontal functions. For example, prefrontal 5-HT depletion in marmosets acts to impair reversal learning, while leaving attentional set-shifting intact (Clarke et al., 2005). Similarly, 5-HT depletion has also been shown to impair performance of a serial discrimination reversal task (Clarke et al., 2004). Although a decrease in prefrontal 5-HT leads to a decrease in cognitive flexibility, it seems as though this may work to increase focused attention (Schmitt et al., 2000). As the DRN is the primary source of prefrontal 5-HT, these projections and their reciprocal descending connections clearly play a role in specific cognitive functions (Briand et al., 2007).

Prefrontal cortex and 5-HT/DA interaction

Recent evidence suggests that 5-HT, in conjunction with DA, comodulates cortical activity and is a prime target of a newer atypical antipsychotic

agent. Early microdialysis work by Iyer and Bradberry (1996) demonstrated that 5-HT application (1–10 μ M) increased extracellular DA in a dose-dependent manner to a greater extent in the PFC than in the striatum. Moreover, the increase in DA release was mediated by 5-HT_{1B/D} receptors and not by 5-HT_{2A/C} or 5-HT₃ receptors. Since then, multiple studies have confirmed that 5-HT₁ receptors increase cortical DA release (Matsumoto et al., 1999; Ichikawa et al., 2001; Díaz-Mataix et al., 2005). Moreover, there is evidence that 5-HT's effects on extracellular DA are concentration dependent.

A study by Díaz-Mataix et al. (2005) showed that reverse dialysis of the 5-HT_{1A} receptor agonist *R*-(–)-2-{4-[(chroman-2-methyl)-amino]-butyl}-1,1-dioxo-benzo-[d]isothiazol one HCl (BAYx3702) in the PFC produces bidirectional effects on DA release, whereby low concentrations increase and high concentrations decrease DA release. The decrease in DA with high 5-HT_{1A} agonist concentration appears to be GABA-mediated, as decreases were not observed when GABA_A receptors were blocked by perfusion with bicuculline. Interestingly, animals given BAYx3702 systemically displayed similar increases in cortical DA release, effects that were blocked following frontocortical transection. Together, these findings suggest that 5-HT_{1A} receptors may be acting on pyramidal glutamatergic PFC cells projecting to the VTA to regulate DA release. Thus, given that 5-HT_{1A} receptors mediate cortical DA release and that atypical drugs, such as clozapine, are weak partial agonists at the 5-HT_{1A} receptors (Assié et al., 2005), it has been suggested that the therapeutic properties of atypical antipsychotic drugs (APDs) may be related to weak 5-HT_{1A} receptor activation in conjunction with D₂ receptor antagonism (Millan, 2000; Meltzer et al., 2003).

Regarding the 5-HT_{2A} receptor, it was shown that a discrete subpopulation of large neurons in the deep layers of the PFC are strongly excited by 5-HT_{2A} receptor activation (Béïque et al., 2007). These findings suggest that cortical 5-HT_{2A} receptors mediate glutamatergic recurrent network activity in the PFC, which is a finding that could be significant, given that these pyramidal cells

may synapse onto mesocortical DA cell bodies in the VTA (Sesack and Pickel, 1992). On the other hand, the systemic administration of (\pm)-2,5-dimethoxy-4-iodoamphetamine (DOI) increases glutamate efflux in the VTA (as well as DA efflux in the PFC), effects that are reversed following intra-PFC infusions with the 5-HT_{2A} receptor antagonist M100907 (Gobert and Millan, 1999; Pehek et al., 2001).

Thus, it appears that the activity of VTA DArgic neurons is under the excitatory control of 5-HT_{2A} receptors in the PFC. Given these findings, Pehek et al. (2006) suggested that 5-HT_{2A} receptor agonists may increase the activity of cortico-tegmental glutamatergic projection neurons, which in turn increase DA release and neuronal activity in the PFC. Hence, cortical 5-HT_{2A} receptors may enhance the overall excitability of PFC networks indirectly via the mesocortical pathway, an effect that may contribute to the positive symptoms of schizophrenia (Di Pietro and Seamans, 2007).

However, like DA, 5-HT's effect on cortical NMDA synaptic currents is bidirectional and concentration dependent. Activation of 5-HT_{2A} receptors can either facilitate or inhibit NMDA-induced responses in the PFC with a low concentrations of 5-HT agonist facilitating NMDA responses, and higher concentrations inhibiting them (Arvanov et al., 1999). In addition to these effects at NMDA receptors, 5-HT_{2A} receptors have been shown to induce weak, but long-lasting enhancements of spontaneous EPSCs in conjunction with large desensitizing enhancements of spontaneous IPSCs (Zhou and Hablitz, 1999).

In this way, APDs (especially those that act as 5-HT_{1A} agonists) may help stabilize cortical activity by enhancing the effect of 5-HT₁ while suppressing spontaneous 5-HT₂-induced excitation. When coupled with the removal of D₂-mediated reductions in NMDA and GABA currents and the potential activation of D₁ receptors by elevated DA levels, atypical APDs should produce strong NMDA and GABA activation, with a reduction in spontaneous EPSCs to evoke the ideal blend of modulation necessary to enhance cortical signal-to-noise ratios and improve cognition as predicted (Winterer and Weinberger, 2004).

In this manner, all the neurochemical and behavioural experimental results inferred from drugs used in the treatment of illnesses, such as depression and schizophrenia, support the existence of modulatory mechanisms involving 5-HT and DA systems as key players. However, experimental studies relating the interactive participation of these neurotransmitter systems in the regulation of prefrontally sustained cognitive processes are at an early stage.

In conclusion, a complex picture is obtained from the data evaluating 5-HT and DA regulation of cognitive processes. From this, an interaction can be inferred on analyzing data from similar tests (similar in regard to cognitive process), but few studies have evaluated this interaction until now. Less is known about the possible modulation of 5-HTergic function by the DA system, despite the evidence that DArgic terminals on RN proceed into the SN (Affi and Kaelber, 1965; Pasquier et al., 1977; Sakai et al., 1977; Lee and Geyer, 1984; Kalén et al., 1988), and the high density of D₂ receptors on raphe neurons (Bouthenet et al., 1987). Future work must address a more integrative approach to the organization of cognition by multiple neurotransmitter systems, because this is the better focus by which significant progress may be made in the development of therapeutic strategies.

Clinical implications of 5-HT/DA interaction

Studies have been focused on DA–5HT interactions as a part of pathophysiological phenomena and therapeutic possibilities dealing with neurological and psychiatric diseases.

This is the case of PD which primarily results from the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) at the origin of the nigrostriatal DArgic pathway. Loss of these DArgic neurons leads to striatal deficiency in DA neurotransmission, accounting for the major symptoms of PD, and supporting the proposal of replenishment of striatal DA through the DA precursor L-DOPA as an effective treatment to alleviate most of the motor symptoms of PD (Dauer and Przedborski, 2003).

Further, a profile of cognitive impairment of PD patients has been identified in relation to the spatio-temporal progression of DA depletion. In the early disease stages, cognitive deficits in PD patients are mainly associated to dorsal striatum dysfunction (impaired adaptation of well-established stimulus–response mappings and reduced updating within working memory). In fact, besides the improvement of motor symptoms, L-DOPA treatment may alleviate dorsal striatum-dependent cognitive alterations (Cools, 2006). However, L-DOPA may affect cognitive functions in PD patients through non-DArgic mechanisms such as reduction of 5-HTergic neurotransmission, as suggested by the similar effects of L-DOPA and 5-HT depletion on some learning processes (Clarke et al., 2004), as well as by the reduction of the content of brain 5-HT elicited by L-DOPA (Kostrzewa et al., 2005), and the opposite interaction between DA and 5-HT in the striatum and in the PFC (Millan et al., 1998; Di Giovanni et al., 2006) which may be relevant for the effects of L-DOPA on cognitive functions in PD patients.

The involvement of disturbances of the cerebral 5-HTergic system in PD has been investigated in human beings both under post-mortem, as well as in vivo experimental designs by using histological, neurochemical and autoradiographic techniques, in view of the possibility that mood and cognitive problems appearing as clinically relevant components of PD, could be explained by disruption of 5-HT neurotransmission including damage to serotonin neurons (Kish, 2003), and because of the relevant role that has been recognized for the 5-HTergic system as a regulator of the functioning of basal ganglia (Di Giovanni et al., 2006).

Degeneration of 5-HTergic neurons in the MRN (Halliday et al., 1990; Paulus and Jellinger, 1991) which could underlie the decreased content in 5-HT, its metabolites and the 5-HT transporter (SERT) in the striatum (Chinaglia et al., 1993; Kerenyi et al., 2003; Kim et al., 2003; Guttman et al., 2007; Kish et al., 2008), the cerebral cortex (Scatton et al., 1983) and the cerebrospinal fluid (Mayeux et al., 1984; Kuhn et al., 1996), as well as alterations on the activities of various 5-HT receptor subtypes (Cheng et al., 1991; Castro et al., 1998), have been demonstrated in PD patients.

The reductions of key markers of striatal 5-HTergic activity support the proposal of a 5-HTergic disturbance in PD. These reductions differentially affecting caudate and putamen (the greater reduction in caudate: 55%, the lesser reduction in putamen: 36%, as compared to healthy subjects), though quantitative individual differences suggest that some PD patients are affected more than others. Besides, a greater susceptibility to damage of raphe nucleus 5-HT neurons innervating the caudate, than those innervating the putamen and the primary involvement of caudate as a target of functional alterations caused by striatal serotonin disruption (possibly cognitive impairment, including cognitive aspects of motor control) could be inferred from these data (Kish et al., 2008), while dysfunction due to putamen 5-HT deficiency might be a secondary pathophysiological component of PD.

It seems also possible that structural damage to raphe-striatal neurons in PD might be limited to the nerve terminal region, as suggested by the significantly reduced densities of SERT-binding sites in the basal ganglia of PD patients (Haapaniemi et al., 2001; Kerényi et al., 2003; Kim et al., 2003), but the normal SERT binding in the dorsal raphe of patients showing decreased SERT binding in the striatum (Chinaglia et al., 1993). The reduction of these 5-HT markers in caudate supports a possible benefit of pharmacological measures aimed to correct the 5-HT deficiency in PD, as suggested by the anti-dyskinetic effect of MDMA in a monkey model of PD (Iravani et al., 2003) which has been explained by the ability of the amphetamine derivative to release 5-HT. In fact, improvement of bradykinesia and finger taps, was observed at 1 and 4 months during treatment with citalopram, a 5-HT reuptake inhibitor, in PD patients, and among them, a clear improvement of mood was observed in 15 of 16 PD patients with depression (Rampello et al., 2002).

The possible functional significance of the reduction of 5-HTergic neurotransmission in these striatal regions is still unclear and deserves further study in PD. As has been hypothesized (Mayeux, 1990), the reduced serotonergic neurotransmission

may result in a compensatory adjustment for the reduced striatal DA activity. In this context, the role of 5-HT in the neuronal substrate forming the SNpc has not yet been well-established, but an inhibitory action of 5-HT over the SNpc neurons, has been recognized, as shown by studies involving the activation of 5-HTergic neurons located at the DRN and microiontophoretic application of 5-HT on SNpc DA neurons (Gervais and Rouillard, 2000); though 5-HT may on the other hand increase the firing rate of SNpc DA neurons in vitro. This inhibitory effect of 5-HT neurotransmission includes the DA release at the striatal nerve endings, as evidenced by the increase of DA release induced by 5HT₂ and 5-HT_{2C} antagonists; while the opposite effect is mediated by 5-HT_{1A} and 5-HT_{2C} receptors (Ugedo et al., 1989; Jacobs and Fornal, 1993; Murphy et al., 1998; Blackburn et al., 2002; Alex et al., 2005; Di Giovanni et al., 2006); as well as by suppression of spontaneous firing in the striatal cells following DRN stimulation. Both, a tonic and a phasic modulation of mesocorticolimbic DA functioning have been shown to be exerted through 5-HT_{2C} receptor subtype (Di Matteo et al., 1998, 2000, 2004; Pierucci et al., 2004).

Human post-mortem tissue from PD patients, have revealed that DA depletion may result in adjustments of 5-HT receptors. While a change in the density of striatal 5-HT_{2C} receptors was not observed, striatal 5-HT_{2A} receptors and 5-HT_{2C} receptors in the substantia nigra pars reticulata appear to be upregulated (Fox and Brotchie, 2000a) in patients with PD. These changes might also be a compensatory consequence of a decreased level of 5-HT in these nuclei and thus, they potentially may be relevant for the neuronal mechanisms involved in PD and for possible pharmacological interventions. In this context, a 5-HT_{2C} antagonist enhances the anti-parkinsonian effect of D₁ and D₂ agonists in a rat model (Fox and Brotchie, 2000b); ritanserin a 5-HT_{2A/2C} receptor antagonist ameliorates exciting extrapyramidal side effects of classical APDs in schizophrenics (Bersani et al., 1990). The reduced motor side-effects during the antipsychotic treatment with clozapine have been ascribed to its 5-HT_{2C} antagonist action which may result in

anti-parkinsonian activity, possibly counteracting the pro-parkinsonian effects of the DA blockade elicited by the APD (Durif et al., 2004).

Cerebral 5-HT activity has also been shown to be involved in cognitive functions, (Buhot et al., 2000) thus, memory consolidation is impaired in healthy human beings as a consequence of ATD (Riedel et al., 1999), a procedure that allows to study in human beings the relationship between cerebral 5-HT activity and mood, cognitive and motor functions in vivo (Booij et al., 2003; Gallagher et al., 2003; Hughes et al., 2003).

When the effects of ATD are assessed in early-stage PD patients, the acute reduction of cerebral 5-HT levels does not affect several parameters of cognitive function (impairment of delayed recall and delayed recognition in the Visual Verbal Learning Task) and motor performance (shortening of reaction times) in a different manner as it does in healthy subjects (Riedel et al., 1999; Scholtissen et al., 2006). Besides, the ATD does not improve PD motor symptoms (Unified Parkinson Disease Rating Scale), as could be expected in view of the proposed inhibitory effects of 5-HT on striatal DA release. In general, the sole effects of ATD in PD patients, neither support a direct role of 5-HT on cognitive and motor functioning other than in healthy subjects, nor a compensatory role of 5-HT adjustments for the nigrostriatal DA activity; though, it could also be expected that distinct short-term and long-term neural mechanisms were involved in the development of motor and cognitive impairments in PD, even if cerebral 5-HT activity is reduced in the early stages of the disease.

Serotonin neurons innervating the striatum (caudate and putamen) in PD patients could be a neural substrate that favours the anti-parkinsonian action of L-DOPA, by assuming that serotonin neurons in human beings can convert exogenous DOPA to DA, as well as store and release the neurotransmitter from their striatal nerve endings as occurs in other species (Tanaka et al., 1999; Maeda et al., 2005). Besides, it has also been discussed (Kish et al., 2008) that if L-DOPA-induced dyskinesias might be caused in part by a drug-induced exaggerated increase in striatal synaptic DA, and 5-HT nerve terminals which

can take up DA from the extracellular space, could be of some benefit by helping to normalize extracellular DA concentration.

However, the above described mechanism for striatal DA synthesis and release from exogenous L-DOPA, has rather been proposed as a possible cause of L-DOPA-induced dyskinesias, given the association between dyskinesias and excessively increased synaptic DA as suggested by PET imaging findings in human PD (De la Fuente-Fernandez et al., 2004; Pavese et al., 2006). In this context, dyskinesias could be explained by deregulated swings of L-DOPA-induced extracellular DA, released from the remaining striatal 5-HT neurons that are unable to normally regulate the DA release. Thus the relative preservation of 5-HTergic function in the putamen would be detrimental to the patient with PD with respect to this L-DOPA adverse effect, a situation in which decreasing striatal 5-HTergic activity by either lesion or pharmacological treatment can actually block L-DOPA-induced dyskinesias in experimental animals (Carta et al., 2007). However SERT binding has shown to be similar in putamen of those clinically advanced PD living patients having more versus less severe drug-induced dyskinesias (Guttman et al., 2007).

The therapeutic success of novel atypical APDs, as shown by their better efficiency against mood alterations and the reduced side effects in schizophrenic patients has been ascribed to drug effects on both 5-HT and DA systems involved or able to positively influence this pathophysiological condition (Alex and Pehek, 2007; Stone and Pilowsky, 2007). Even though an improvement of mood negative symptoms and behavioural alterations has been the main objective of APDs clinical studies (Kapur and Remington, 1996; Truffinet et al., 1999; Bandelow and Meier, 2003; Iwakawa et al., 2004; Werkman et al., 2006; Mamo et al., 2007), attention has also been paid to the effects of these drugs on the cognitive dysfunctions of schizophrenic patients (Araki et al., 2006; Di pietro and Seamans, 2007; Gray and Roth, 2007; Scholes et al., 2007). Different aspects of cognitive functions are shown to be affected under APDs treatment, depending on the chosen drug, the duration of treatment, and on specific individual

characteristics of the disease. Thus, attention, psychomotor, speed-executive skills, working memory and spatial memory, verbal memory are among other cognitive functions differentially affected by APDs.

Both DArgic and serotonergic mechanisms have been identified in the effects of the abuse drug 3-4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') on the CNS (Green et al., 2003). However, cognitive impairment elicited by MDMA, has rather been ascribed to reduced serotonergic activity, due to permanent neurotoxic brain damage (Reneman et al., 2000; Gouzoulis-Mayfrank et al., 2000, 2003; Colado et al., 2004; Able et al., 2006; Quednow et al., 2006; Hoshi et al., 2007; Zakzanis et al., 2007) which may remain for years (Ward et al., 2006).

Although the complete clinical significance of DA-serotonin interactions could be addressed by future in vivo studies in human beings, it is well-recognized today that cognitive dysfunction in neurological and psychiatric diseases, as well as under some drug abuse conditions, in which neurotransmitter impairments are involved, account for long-term disability, especially difficult to be treated, and being, in some patients the main factors responsible for a bad quality of life.

Abbreviations

AAPA	active allothetic place avoidance
AN	accumbens nucleus
AS19	(2 <i>S</i>)-(+) -5-(1,3,5-trimethylpyrazol-4-yl)-2-(dimethylamino) tetralin
A69024	1-[(2-bromo-4,5- dimethoxyphenyl) methyl]-1, 2,3,4-tetrahydro-6-methoxy-2-methyl-7-isoquinolinol
A77636	((1 <i>R</i> - <i>cis</i>)-1-(aminomethyl)-3,4-dihydro-3-tricyclo[3.3.1.1 ^{3,7}]dec-1-yl-[1H]-2-benzopyran-5,6-diol hydrochloride)
BAYx3702	<i>R</i> -(-)-2-[4-[(chroman-2-methyl)-amino]-butyl]-1,1-dioxo-benzo-[d]isothiazol one HCl
BMN	basal magnocellular nucleus
CG1	cingulated cortex 1

DA	dopamine
DOI	(±)-2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine
DRN	dorsal raphe nucleus
DSP4	<i>N</i> -(2-chloroethyl)- <i>N</i> -ethyl-2-bromobenzylamine
EPSCs	excitatory post-synaptic currents
GABA	gamma-aminobutyric acid
GR125487	[1-[2(methylsulfonyl)amino]ethyl]-4-piperidinyl] methyl-5-fluoro-2-methoxy-1 <i>H</i> -indole-3-carboxylate
ICV	intracerebroventricular
IL	infralimbic cortex
IPSCs	inhibitory post-synaptic currents
IP3	inositol phosphate
LTD	long term depression
LTM	long term memory
LTP	long term potentiation
L745,870	3-(4-[4-Chlorophenyl] piperazin-1-yl)-methyl-1 <i>H</i> -pyrrolo[2,3- <i>b</i>]pyridine trihydrochloride
mCPBG	<i>m</i> -chlorophenylbiguanide
MDA	(+ / -)-3,4-(methylenedioxy)amphetamine
MDMA	methylenedioxymethamphetamine
MPTP	1-methyl-1-4-phenyl-1,2,3,6-tetrahydropyridine
MRN	medial raphe nucleus
M100907	[<i>R</i> -(+)-δ-(2,3-dimethoxyphenyl)-1-[4-fluorophenylethyl]-4-piperidinemethanol]
NA	noradrenaline
NAN-190	1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide
NAD-229	2 <i>H</i> -1-benzopyran-5-carboxamide
NMDA	<i>N</i> -methyl-D-aspartate
PCA	<i>p</i> -chloroamphetamine
PCPA	<i>p</i> -chlorophenylalanine
PFC	prefrontal cortex
PL	prelimbic cortex
RN	raphe nuclei
RO4368554	(3-benzenesulfonyl-7-(4-methylpiperazine-1-yl)- <i>H</i> -indole

SB242084	6-chloro-5-methyl-1-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl]indoline
SB269970	(<i>R</i>)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl)-phenol
SCH23390	((<i>R</i>)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5,-tetrahydro-1H-3-benzazepine hydrochloride.
SDZ 205557	(2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester hydrochloride
SKF82958	[(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide]
SL65.0155	(5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one-mono-hydrochloride)
SN	substantia nigra
STM	short-term memory
Trp	tryptophan
VTA	ventral tegmental area
WAY-100635	<i>N</i> -[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]- <i>N</i> -(2-pyridinyl)cyclohexane carboxamide trihydrochloride
5-HT	serotonin
5,7-DHT	5,7-dihydroxytryptamine
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2(di- <i>N</i> -propylamino)-tetralin

References

- Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., Huang, Y., Hwang, D.R., Keilp, J., Kochan, L., Van Heertum, R., Gorman, J.M. and Laruelle, M. (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J. Neurosci.*, 22(9): 3708–3719.
- Able, J.A., Gudelsky, G.A., Vorhees, C.V. and Williams, M.T. (2006) 3,4-methylenedioxymethamphetamine in adult rats produces deficits in path integration and spatial reference memory. *Biol. Psychiatry*, 59(12): 1219–1226.
- Abrahams, B.S., Rutheford, J.D., Mallet, P.E. and Beninger, R.J. (1998) Place conditioning with the dopamine D1-like receptor agonist SKF 8295 but not SKF 81297 or SKF 77434. *Eur. J. Pharmacol.*, 343(2–3): 111–118.
- Afifi, A. and Kaelber, W.W. (1965) Efferent connections of the substantia nigra in the cat. *Exp. Neurol.*, 11: 474–482.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113(2): 296–320.
- Alex, K.D., Yavarian, G.J., McFarlane, H.G., Pluto, C.P. and Pehek, E.A. (2005) Modulation of dopamine release by striatal 5-HT_{2C} receptors. *Synapse*, 55(4): 242–251.
- Allan, A.M., Galindo, R., Chynowet, J., Engel, S.R. and Savage, D.D. (2001) Conditioned place preference for cocaine is attenuated in mice over-expressing the 5-HT(3) receptor. *Psychopharmacol. (Berl.)*, 158(1): 18–27.
- Altman, H.J., Normile, H.J., Galloway, M.P., Ramirez, A. and Azmitia, E.C. (1990) Enhanced spatial discrimination learning in rats following 5,7-DHT-induced serotonergic deafferentation of the hippocampus. *Brain Res.*, 518(1–2): 61–66.
- Amaral, D.G. and Price, J.L. (1984) Amygdalo-cortical projections in the monkey (*Macaca fascicularis*). *J. Comp. Neurol.*, 230(4): 465–496.
- Anguiano-Rodríguez, P., Gaytán-Tocavén, L. and Olvera-Cortés, M.E. (2007) Striatal serotonin depletion facilitates rat egocentric learning via dopamine modulation. *Eur. J. Pharmacol.*, 556(1–3): 91–98.
- Araki, T., Yamasue, H., Sumiyoshi, T., Kuwabara, H., Suga, M., Iwanami, A., Kato, N. and Kasai, K. (2006) Perospirone in the treatment of schizophrenia: effect on verbal memory organization. *Progr. Neuropsychopharmacol. Biol. Psychiatry*, 30(2): 204–208.
- Arvanov, V.L., Liang, X., Magro, P., Roberts, S. and Wang, R.Y. (1999) A pre- and post-synaptic modulatory action of 5-HT and 5-HT_{2A}, 2C receptor agonist DOB on NMDA-evoked responses in the rat medial prefrontal cortex. *Eur. J. Neurosci.*, 11(8): 2917–2934.
- Asin, K.E. and Fibiger, H.C. (1984) Spontaneous and delayed spatial alternation following damage to specific neuronal elements within the nucleus medianus raphe. *Behav. Brain Res.*, 13(3): 241–250.
- Assié, M.B., Ravhaile, V., Faucillon, V., Newman-Tancredi, A. (2005) Contrasting contribution of 5-hydroxytryptamine 1A receptor activation to neurochemical profile of novel antipsychotics: frontocortical dopamine and hippocampal serotonin release in rat brain. 315(1): 265–272.
- Aultman, J.M. and Moghaddam, B. (2001) Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. *Psychopharmacol. (Berl.)*, 153(3): 353–364.
- Azmitia, E.C. and Segal, M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.*, 179(3): 641–667.
- Badgaiyan, R.D., Fischman, A.J. and Alpert, N.M. (2007) Striatal dopamine release in sequential learning. *Neuroimage*, 38(3): 549–556.

- Bandelow, B. and Meier, A. (2003) Aripiprazole, a "dopamine-serotonin system stabilizer" in the treatment of psychosis. *Ger. J. Psychiatry*, 6(1): 9–16.
- Barbas, H. and De Olmos, J. (1990) Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *J. Comp. Neurol.*, 300(4): 549–571.
- Barbas, H. and Pandya, D.N. (1989) Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *J. Comp. Neurol.*, 286(3): 353–375.
- Barnes, J.M., Costall, B., Coughlan, J., Domeney, A.M., Gerrard, P.A., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tomkins, D.M. and Tyers, M.B. (1990) The effects of ondansetron, a 5-HT₃ receptor antagonist, on cognition in rodents and primates. *Pharmacol. Biochem. Behav.*, 35(4): 955–962.
- Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8): 1083–1152.
- Beart, P.M. and McDonald, D. (1982) 5-Hydroxytryptamine 5-hydroxytryptaminergic-dopaminergic interactions in the ventral area of rat brain. *J. Pharm. Pharmacol.*, 34(9): 591–593.
- Béïque, J.C., Imad, M., Mladenovic, L., Gingrich, J.A. and Andrade, R. (2007) Mechanism of the 5-hydroxytryptamine 2A receptor-mediated facilitation of synaptic activity in prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 104(23): 9870–9875.
- Bersani, G., Grispi, A., Marini, S., Pasini, A., Valducci, M. and Ciani, N. (1990) 5-HT₂ antagonist ritanserin in neuroleptic-induced parkinsonism: a double-blind comparison with orphenadrine and placebo. *Clin. Neuropharmacol.*, 13(6): 500–506.
- Biggio, G., Fadda, F., Fanni, P., Tagliamonte, A. and Gessa, G.L. (1974) Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindoleacetic acid by a tryptophan-free diet. *Life Sci.*, 14(7): 1321–1329.
- Blackburn, T.P., Minabe, Y., Middlemiss, D.N., Shirayama, Y., Hashimoto, K. and Ashby, C.R., Jr. (2002) Effect of acute and chronic administration of the selective 5-HT_{2C} receptor antagonist SB-243213 on midbrain dopamine neurons in the rat: an in vivo extracellular single cell study. *Synapse*, 46(3): 129–139.
- Blandina, P., Goldfarb, J., Craddock-Royal, B. and Green, J.P. (1989) Release of endogenous dopamine by stimulation of 5-hydroxytryptamine₃ receptors in rat striatum. *J. Pharmacol. Exp. Ther.*, 251(3): 803–809.
- Booij, L., Van der Does, A.J. and Riedel, W.J. (2003) Monoamine depletion in psychiatric and healthy populations. *Mol. Psychiatry*, 8(12): 951–973.
- Boulougouris, V., Glennon, J.C. and Robbins, T.W. (2008) Dissociable effects of selective 5-HT_{2A} and 5-HT_{2C} receptor antagonists on serial spatial reversal learning in rats. *Neuropsychopharmacology*, 33(2): 2007–2019.
- Bourson, A., Boess, F.G., Börs, M. and Sleight, A.J. (1998) Involvement of 5-HT₆ receptors in nigro-striatal function in rodents. *Br. J. Pharmacol.*, 125(7): 1562–1566.
- Bouthenet, M.L., Martres, M.P., Sales, N. and Schwartz, J.C. (1987) A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [¹²⁵I]iodosulpride. *Neuroscience*, 20(1): 117–155.
- Brasted, P.J., Humby, T., Dunnett, S.B. and Robbins, T.W. (1997) Unilateral lesions of the dorsal striatum in rats disrupt responding in egocentric space. *J. Neurosci.*, 17(22): 8919–8926.
- Briand, L.A., Gritton, H., Howe, W.M., Young, D.A. and Sarter, M. (2007) Modulators in concert for cognition: modulator interactions in the prefrontal cortex. *Prog. Neurobiol.*, 83(2): 69–91.
- Brodmann, K. (1909) Vergleichende lokalisationslehre der grosshirnhinde. Barth, Leipzig.
- Buhot, M.C. (1997) Serotonin receptors in cognitive behaviors. *Curr. Opin. Neurobiol.*, 7(2): 243–254.
- Buhot, M.C., Martin, S. and Segu, L. (2000) Role of serotonin in memory impairment. *Ann. Med.*, 31(3): 210–221.
- Buhot, M.C., Patra, S.K. and Naïli, S. (1995) Spatial memory deficits following stimulation of hippocampal 5-HT_{1B} receptors in the rat. *Eur. J. Pharmacol.*, 285(3): 218–221.
- Bubser, M. and Schmidt, W.J. (1990) 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav. Brain Res.*, 37(2): 157–168.
- Calabresi, P., Pisani, A., Mercuri, N.B. and Bernardi, G. (1992) Long-term potentiation in the striatum is unmasked by removing the voltage-dependent blockade of NMDA receptor channel. *Eur. J. Neurosci.*, 4(10): 929–935.
- Campbell, A.D., Kohl, R.R. and McBride, W.J. (1996) Serotonin-3 receptor and ethanol-stimulated somatodendritic dopamine release. *Alcohol*, 13(6): 569–574.
- Carbon, M.A., Ma, Y., Barnes, A., Dhawan, V., Chaly, T., Ghilardi, M.F. and Eidelberg, D. (2004) Caudate nucleus: influence of dopaminergic input on sequence learning and brain activation in Parkinsonism. *Neuroimage*, 21(4): 1497–1507.
- Carli, M., Luschi, R., Garofalo, P. and Samanin, R. (1995) 8-OH-DPAT impairs spatial but not visual learning in a water maze by stimulating 5-HT_{1A} receptors in the hippocampus. *Behav. Brain Res.*, 67(1): 67–74.
- Carli, M., Silva, S., Balducci, C. and Samanin, R. (1999) WAY 100635, a 5-HT_{1A} receptor antagonist, prevents the impairment of spatial learning caused by blockade of hippocampal NMDA receptors. *Neuropharmacology*, 38(8): 1165–1173.
- Carta, M., Carlsson, T., Kirik, D. and Bjökund, A. (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain*, 130(7): 1819–1833.
- Castellano, C., Cestari, V., Cabib, S. and Puglisi-Allegra, S. (1991) Post-training dopamine receptor agonists and antagonists affect memory storage in mice irrespective of their selectivity for D₁ and D₂ receptors. *Behav. Neural Biol.*, 56(3): 283–291.
- Castro, M.E., Pascual, J., Romon, T., Bercianno, J., Figols, J. and Pazos, A. (1998) 5-HT_{1B} receptor binding in degenerative movement disorders. *Brain Res.*, 790(1–2): 323–328.

- Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G. and Artigas, F. (2001) Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABA(A), and glutamate receptors. *J. Neurosci.*, 21(24): 9917–9929.
- Chapman, D.E., Hanson, G.R., Kesner, R.P. and Keefe, K.A. (2001) Long-term changes in basal ganglia function after a neurotoxic regimen of methamphetamine. *J. Pharmacol. Exp. Ther.*, 296(2): 520–527.
- Chen, G., Greengard, P. and Yan, Z. (2004) Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 101(8): 2596–2600.
- Cheng, A.V., Ferrier, I.N., Morris, C.M., Jabeen, S., Shagal, A., McKeith, I.G., Edwardson, J.A., Pery, R.H. and Perry, E.K. (1991) Cortical serotonin-S2 receptor binding in Lewy body dementia, Alzheimer's and Parkinson's diseases. *J. Neurol. Sci.*, 106(1): 50–55.
- Chinaglia, G., Landwehrmeyer, B., Probst, A. and Palacios, J.M. (1993) Serotonergic terminal transporters are differentially affected in Parkinson's disease and progressive nuclear palsy: an autoradiographic study with [3H]citalopram. *Neuroscience*, 54(3): 691–699.
- Cimadevilla, J.M., Wesierska, M., Fenton, A.A. and Bures, J. (2001) Inactivating one hippocampus impairs avoidance of a stable room-defined place during dissociation of arena cues from room cues by rotation of the arena. *Proc. Natl. Acad. Sci. U.S.A.*, 98(6): 3531–3536.
- Clarke, H.F., Dalley, J.W., Crofts, H.S., Robbins, T.W. and Roberts, A.C. (2004) Cognitive inflexibility after prefrontal serotonin depletion. *Science*, 304(5672): 878–880.
- Clarke, H.F., Walker, S.C., Crofts, H.S., Dalley, J.W., Robbins, T.W. and Roberts, A.C. (2005) Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J. Neurosci.*, 25(2): 532–538.
- Colado, M.I., O'Shea, E. and Green, A.R. (2004) Acute and long-term effects of NDMA on cerebral dopamine biochemistry and function. *Psychopharmacology*, 173(3–4): 249–263.
- Cools, R. (2006) Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease. *Neurosci. Biobehav. Rev.*, 30(1): 1–23.
- Daberkow, D.P., Kesner, R.P. and Keefe, K.A. (2005) Relation between methamphetamine-induced monoamine depletions in the striatum and sequential motor learning. *Pharmacol. Biochem. Behav.*, 81(1): 198–204.
- Dauer, W. and Przedborski, S. (2003) Parkinson disease: mechanisms and models. *Neuron*, 39(6): 889–909.
- Daw, N.D., Kakade, S. and Dayan, P. (2002) Opponent interactions between serotonin and dopamine. *Neural Netw.*, 15(4–6): 603–616.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2003) Potentiation of amphetamine-induced changes in dopamine and 5-HT by a 5-HT(6) receptor antagonist. *Brain Res. Bull.*, 59(6): 513–521.
- De Belleruche, J.S. and Bradford, H.F. (1980) Presynaptic control of the synthesis and release of dopamine from striatal synaptosomes: a comparison between the effects of 5-hydroxytryptamine, acetylcholine, and glutamate. *J. Neurochem.*, 35(5): 1227–1234.
- DeCoteau, W.E. and Kesner, R.P. (2000) A double dissociation between the rat hippocampus and medial caudoputamen in processing two forms of knowledge. *Behav. Neurosci.*, 114(6): 1096–1108.
- De la Fuente-Fernandez, R., Sois, V., Huang, Z., Furatado, S., Lu, J.Q., Calne, D.B., Ruth, T.J. and Stoessl, A.J. (2004) Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. *Brain*, 127(pt. 12): 2747–2754.
- De Simoni, M.G., Dal Toso, G., Fodritto, F., Sokola, A. and Algeri, S. (1987) Modulation of striatal dopamine metabolism by the activity of dorsal raphe serotonergic afferences. *Brain Res.*, 411(1): 81–88.
- Diaz-Mataix, L., Scorza, M.C., Bortolozzi, A., Toth, M., Celada, P. and Artigas, F. (2005) Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *J. Neurosci.*, 25(47): 10831–10843.
- Di Giovanni, G., De Dewaerdere, P., Di Mascio, M., Di Matteo, V., Esposito, E. and Spampinato, U. (1999) Selective blockade of serotonin-2B/2C receptors enhances mesolimbic and mesostriatal dopaminergic function: A combined in vivo electrophysiological and microdialysis study. *Neuroscience*, 91(2): 587–597.
- Di Giovanni, G., Di Matteo, V., Di Mascio, M. and Esposito, E. (2000) Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. *Synapse*, 35(1): 53–61.
- Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A. and Espósito, E. (2006) Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT2C receptor be a new target for therapeutic strategies? *Curr. Med. Chem.*, 13(25): 3069–3081.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Espósito, E. (1998) Selective blockade of serotonin 2C/2B receptors enhances dopamine release in the rat nucleus accumbens. *Neuropharmacology*, 37(2): 265–272.
- Di Matteo, V., Di Giovanni, G., Di Mascio, M. and Espósito, E. (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin (2C) receptors. *Brain Res.*, 865(1): 85–90.
- Di Matteo, V., Pierucci, M. and Espósito, E. (2004) Selective stimulation of serotonin 2c receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine. *J. Neurochem.*, 89(2): 418–429.
- Di Pietro, N.C. and Seamans, J.K. (2007) Dopamine and serotonin interactions in the prefrontal cortex: insights on antipsychotic drugs and their mechanism of action. *Pharmacopsychiatry*, 40(Suppl. 1): S27–S33.
- Dunnett, S.B. and White, A. (2006) Striatal grafts alleviate bilateral striatal lesion deficits in operant delayed alternation in the rat. *Exp. Neurol.*, 199(2): 479–489.
- Durif, F., Debilly, B., Galitzky, M., Morand, D., Viallet, F., Borg, M., Thobois, S., Broussolle, E. and Rascol, O. (2004)

- Clozapine improves dyskinesias in Parkinson disease: a double-blind, placebo-controlled study. *Neurology*, 62(3): 381–388.
- Durstewitz, D. and Seamans, J.K. (2002) The computational role of dopamine D1 receptors in working memory. *Neural Netw.*, 15(4–6): 561–572.
- Durstewitz, D. and Seamans, J.K. (2006) The computational role of dopamine D1 receptors in working memory. *Neural Netw.*, 15(4–6): 561–572.
- Egashira, N., Yano, A., Ishigami, N., Mishima, K., Iwasaki, K., Fujioka, M., Matsushita, M., Nishimura, R. and Fujiwara, M. (2006) Investigation of mechanisms mediating 8-OH-DPAT-induced impairment of spatial memory: involvement of 5-HT_{1A} receptors in the dorsal hippocampus of rats. *Brain Res.*, 1069(1): 54–62.
- Eglen, R.M., Jasper, J.R., Chang, D.J. and Martin, G.R. (1997) The 5-HT₇ receptor: orphan found. *Trends. Pharmacol. Sci.*, 18(4): 104–107.
- Eglen, R.M., Wong, E.H., Dumius, A. and Bockaert, J. (1995) Central 5-HT₄ receptors. *Trends Pharmacol. Sci.*, 16(11): 391–398.
- Faure, A., Haberland, U., Condé, F. and Massiou, N.E. (2005) Lesion to the nigrostriatal dopamine system disrupts stimulus–response habit formation. *J. Neurosci.*, 25(11): 2771–2780.
- Featherstone, R.E. and McDonald, R.J. (2004) Dorsal striatum and stimulus–response learning: lesions of the dorsolateral, but not dorsomedial, striatum impair acquisition of a simple discrimination task. *Behav. Brain. Res.*, 150(1–2): 15–23.
- Fernández-Pérez, S., Pache, D.M. and Sewell, R.D. (2005) Co-administration of fluoxetine and WAY100635 improves short-term memory function. *Eur. J. Pharmacol.*, 522(1–3): 78–83.
- Ferré, S., Cortés, R. and Artigas, F. (1994) Dopaminergic regulation of the serotonergic raphe-striatal pathway: microdialysis studies in freely moving rats. *J. Neurosci.*, 14(8): 4839–4846.
- Finlay, J.M., Zigmond, M.J. and Abercrombie, E.D. (1995) Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. *Neuroscience*, 64(3): 619–628.
- Fino, E., Glowinski, J. and Venance, L. (2005) Bidirectional activity-dependent plasticity at corticostriatal synapses. *J. Neurosci.*, 25(49): 11279–11287.
- Fletcher, P.J., Korth, K.M. and Chambers, J.W. (1999) Selective destruction of brain serotonin neurons by 5,7-dihydroxytryptamine increases responding for a conditioned reward. *Psychopharmacol. (Berl.)*, 147(3): 291–299.
- Florio, T., Capozzo, A., Nisini, A., Lupi, A. and Scarnati, E. (1999) Dopamine denervation of specific striatal sub-regions differentially affects preparation and execution of delayed response task in the rat. *Behav. Brain. Res.*, 104(1–2): 51–62.
- Fluxe, K., Hökfelt, T., Johansson, O., Jonsson, G., Lidbrink, P. and Ljungdahl, A. (1974) The origin of the dopamine nerve terminals in limbic and frontal cortex: evidence for meso-cortico dopamine neurons. *Brain Res.*, 82(2): 349–355.
- Fox, S.H. and Brotchie, J.M. (2000a) 5-HT_{2C} receptor binding is increased in the substantia nigra pars reticulata in Parkinson's disease. *Mov. Disord.*, 15(6): 1064–1069.
- Fox, S.H. and Brotchie, J.M. (2000b) 5-HT_{2C} receptor antagonists enhance the behavioural response to dopamine D(1) receptor agonists in the 6-hydroxydopamine-lesioned rat. *Eur. J. Pharmacol.*, 398(1): 59–64.
- Frantz, K.J., Hansson, K.J., Stouffer, D.G. and Parsons, L.H. (2002) 5-HT₆ receptor antagonism potentiates the behavioral and neurochemical effects of amphetamine but not cocaine. *Neuropharmacology*, 42(2): 170–180.
- Fuster, J. (1980) *The Prefrontal Cortex*. Raven, New York.
- Fuster, J.M. (1997) *The prefrontal cortex: anatomy, physiology and neuropsychology of the frontal lobe* (3rd edn.). Lippincott-Raven, Philadelphia.
- Fuster, J.M. (2001) The prefrontal cortex- an update: time is of the essence. *Neuron*, 30(2): 319–333.
- Galeotti, N., Gherardini, C. and Bartolini, A. (1998) Role of 5-HT₄ receptors in the mouse passive avoidance test. *J. Pharmacol. Exp. Ther.*, 286(3): 1115–1121.
- Gallagher, P., Massey, A.E., Young, A.E., et al. (2003) Effects of acute tryptophan depletion on executive function in healthy male volunteers. *Psychiatry*, (3): 1–9.
- Gasbairri, A., Cifariello, A., Pompili, A. and Meneses, A. (2008) Effect of 5-HT₇ antagonist SB-269970 in the modulation of working and reference memory in the rat. *Behav. Brain Res.*, doi:10.1016/j.bbr.2007.12.020.
- Gerard, C., Martres, M.P., Lefevre, K., Miquel, M.C., Verge, D., Lanfumey, L., Doucet, E., Hamon, M. and el Mestikawy, S. (1997) Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.*, 764(1–2): 207–219.
- Gervais, J. and Rouillard, C. (2000) Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and substantia nigra. *Synapse*, 35(4): 281–291.
- Geyer, M.A., Puerto, A., Dawsey, W.J., Knapp, S., Bullard, W.P. and Mandell, A.J. (1976) Histologic and enzymatic studies of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.*, 106(2): 241–256.
- Gobert, A. and Millan, M.J. (1999) Serotonin (5-HT)_{2A} receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology*, 38(2): 315–317.
- Gobert, A., Rivet, J.M., Lejeune, F., Newman-Tancredi, A., Adhumeau-Auclair, A., Nicolas, J.P., Cistarelli, L., Melon, C. and Millan, M.J. (2000) Serotonin(2C) receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. *Synapse*, 36(3): 205–221.
- Goldman-Rakic, P.S. (1987) Circuitry of Primate Prefrontal Cortex and Regulation of Behavior by Representational Memory. *American Physiological Behavior*, Bethesda.
- Goldman-Rakic, P.S. (1995) Cellular basis of working memory. *Neuron*, 14(3): 477–485.
- Goldman-Rakic, P.S., Lidow, M.S. and Gallager, D.W. (1990) Overlap of dopaminergic, adrenergic, and serotonergic

- receptors and complementarity of their subtypes in primate prefrontal cortex. *J. Neurosci.*, 10(7): 2125–2138.
- Goldman-Rakic, P.S., Muly, E.C., III and Williams, G.V. (2000) D(1) receptors in prefrontal cells and circuits. *Brain Res. Rev.*, 31(2–3): 295–301.
- Goldman-Rakic, P.S. and Schwartz, M.L. (1982) Interdigitation of contralateral and ipsilateral columnar projections to frontal association cortex in primates. *Science*, 216(4547): 755–757.
- González-Burgos, G., Kroener, S., Seamans, J.K., Lewis, D.A. and Barrionuevo, G. (2005) Dopaminergic modulation of short-term synaptic plasticity in fast-spiking interneurons of primate dorsolateral prefrontal cortex. *J. Neurophysiol.*, 94(6): 4168–4177.
- González-Burgos, I., Pérez-Vega, M.I., Del Angel-Meza, A.R. and Feria-Velasco, A. (1998) Effect of tryptophan restriction on short-term memory. *Physiol. Behav.*, 63(2): 165–169.
- Gorelova, N., Seamans, J.K. and Yang, C.R. (2002) Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J. Neurophysiol.*, 88(6): 3150–3166.
- Gorelova, N. and Yang, C.R. (2000) Dopamine D1/D5 receptor activation modulates a persistent sodium current in rat prefrontal cortical neurons in vitro. *J. Neurophysiol.*, 84(1): 75–87.
- Gouzoulis-Mayfrank, E., Daumann, J., Tutchenhagen, F., Pelz, S., Becker, S., Kunert, H.-J., Fimm, B. and Sass, H. (2000) Impaired cognitive performance in drug free users of recreational ecstasy (NDMA). *J. Neurol. Neurosurg. Psychiatry*, 68(6): 719–725.
- Gouzoulis-Mayfrank, E., Thimm, B., Rezk, M., Hensen, G. and Daumann, J. (2003) Memory impairment suggests hippocampal dysfunction in abstinent ecstasy users. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27(5): 819–827.
- Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, 41(1): 1–24.
- Grace, A.A. (2000) The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction*, 95(Suppl. 2): S119–S128.
- Gray, J.A. and Roth, B.L. (2007) Molecular targets for treating cognitive dysfunction in schizophrenia. *Schizophr. Bull.*, (5): 1100–1119.
- Graybiel, A.M., Hirsch, E.C., Agid, Y. (1990) The nigrostriatal system in Parkinson's disease. *Adv. Neurol.*, 153, 17–29.
- Green, A.R., Mechan, A.O., Elliot, M., O'Shea, E. and Colado, M.I. (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (NDMA, 'Ecstasy'). *Pharmacol. Rev.*, 55(3): 463–508.
- Groenewegen, H.J. and Uylings, H.B. (2000) The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog. Brain Res.*, 126: 3–28.
- Gu, Q. (2002) Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. *Neuroscience*, 111(4): 815–835.
- Guttman, M., Boileau, I., Warsh, J., Saint-Cyr, J.A., Ginovart, N., McCluskey, T., Houle, S., Wilson, A., Mundo, E., Rusjan, P., Meyer, J. and Kish, S.J. (2007) Brain serotonin transporter binding in non-depressed patients with Parkinson's disease. *Eur. J. Neurol.*, 14(5): 523–528.
- Haapaniemi, T.H., Ahonen, A., Torniainen, P., Sotaniemi, K.A. and Myllylä, V.V. (2001) [123I] Beta-CIT SPECT demonstrates decreased brain dopamine and serotonin transporter levels in untreated parkinsonian patients. *Mov. Disord.*, 16(1): 124–130.
- Halliday, G.M., Blumbergs, P.C., Cotton, R.G.H., Blesing, W.W. and Geffen, L.B. (1990) Loss of brainstem serotonin and substance P-containing neurons in Parkinson's disease. *Brain Res.*, 510(1): 104–107.
- Harrell, A.V. and Allan, A.M. (2003) Improvements in hippocampal-dependent learning and decremental attention in 5-HT(3) receptor overexpressing mice. *Learn. Mem.*, 10(5): 410–419.
- Harrison, A.A., Everitt, B.J. and Robbins, T.W. (1997) Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharmacol. (Berl.)*, 133(4): 329–342.
- Harrison, A.A., Everitt, E.J. and Robbins, T.W. (1999) Central serotonin depletion impairs both the acquisition and performance of a symmetrically reinforced go/no-go conditional visual discrimination. *Behav. Brain Res.*, 100(1–2): 99–112.
- Harvey, J.A. (1996) Serotonergic regulation of associative learning. *Behav. Brain Res.*, 73(1–2): 47–50.
- Hervé, D., Simon, H., Blanc, G., Lisoprawski, A., Le Moal, M., Glowinski, J. and Tassin, J.P. (1979) Increased utilization of dopamine in the nucleus accumbens but not in the cerebral cortex after dorsal raphe lesion in the rat. *Neurosci. Lett.*, 15(2–3): 127–133.
- Hong, E. and Meneses, A. (1996) Systemic injection of *p*-chloroamphetamine eliminates the effect of the 5-HT₃ compounds on learning. *Pharmacol. Biochem. Behav.*, 53(4): 765–769.
- Hoshi, R., Mullins, K., Boundy, C., Brignell, C., Piccini, P. and Curran, H.V. (2007) Neurocognitive function in current and ex-users of ecstasy in comparison to both matched polydrug-using controls and drug-naïve controls. *Psychopharmacol. (Berl.)*, 194(3): 371–379.
- Hritcu, L., Clicinschi, M. and Nabeshima, T. (2007) Brain serotonin depletion impairs short-term memory, but not long-term memory in rats. *Physiol. Behav.*, 91(5): 652–657.
- Hudzik, T.J., Howell, A., Georger, M.M. and Cross, A.J. (2000) Disruption of acquisition and performance of operant response-duration differentiation by unilateral nigrostriatal lesions. *Behav. Brain Res.*, 114(1–2): 65–77.
- Hughes, J.H., Gallagher, P., Stewart, M.E., Mattheus, D., Kelly, T.P. and Young, A.H. (2003) The effects of acute tryptophan depletion on neuropsychological function. *J. Psychopharmacol.*, 17(3): 300–309.
- Hutson, P.H., Barton, C.L., Jay, M., Blurton, P., Burkamp, F., Clarkson, R. and Bristow, L.J. (2000) Activation of

- mesolimbic dopamine function by phencyclidine is enhanced by 5-HT(2C/2B) receptor antagonists: neurochemical and behavioural studies. *Neuropharmacology*, 39(12): 2318–2328.
- Ichikawa, J., Ishii, H., Bonaccorso, S., Fowler, W.L., O'Laughlin, I.A. and Meltzer, H.Y. (2001) 5-HT(2A) and D(2) receptor blockade increases cortical DA release via 5-HT(1A) receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.*, 76(5): 1521–1531.
- Ikemoto, S. and Panksepp, J. (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res. Brain Res. Rev.*, 31(1): 6–41.
- Inoue, T., Izumi, T., Maki, Y., Muraki, I. and Koyama, T. (2000) Effect of dopamine D(1/5) antagonist SCH 23390 on the acquisition of conditioned fear. *Pharmacol. Biochem. Behav.*, 66(3): 573–578.
- Iravani, M.M., Jackson, M.J., Kuoppamäki, M., Smith, L.A. and Jenner, P. (2003) 3,4-methylenedioxymphetamine (ecstasy) inhibits dyskinesia expression and normalizes motor activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates. *J. Neurosci.*, 23: 9107–9115.
- Isayama, S., Sugimoto, Y., Nishiga, M. and Kamei, C. (2001) Effects of histidine on working memory deficits induced by 5-HT1A receptor agonist 8-OH-DPAT. *Jpn. J. Pharmacol.*, 86(4): 451–453.
- Iyer, R.N. and Bradberry, C.W. (1996) Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 277(1): 40–47.
- Iversen, S.D. (1984) 5-HT and anxiety. *Neuropharmacology*, 23(12B): 1553–1560.
- Iwakawa, M., Terao, T., Soya, A., Kojima, H., Inoue, Y., Ueda, N., Yoshimura, R. and Nakamura, J. (2004) A novel antipsychotic, prospirone, has antiserotonergic and antidopaminergic effects in human brain: findings from neuroendocrine challenge tests. *Psychopharmacology*, 176(3–4): 407–411.
- Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.*, 72(1): 165–229.
- Jacobs, B.L. and Fornal, C.A. (1993) 5-HT and motor control: a hypothesis. *Trends Neurosci.*, 16(1): 346–352.
- Jakab, R.L. and Goldman-Rakic, P.S. (2000) Segregation of serotonin 5-HT2A and 5-HT3 receptors in inhibitory circuits of the primate cerebral cortex. *J. Comp. Neurol.*, 417(3): 337–348.
- Jay, T.M. (2003) Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. *Prog. Neurobiol.*, 69(6): 375–390.
- Kaczmarek, L.K. and Levitan, I.B. (1987) *Neuromodulation: the biochemical control of neuronal excitability*. Oxford University Press, New York.
- Kalén, P., Skagerberg, G. and Lindvall, O. (1988) Projections from the ventral tegmental area and mesencephalic raphe to the dorsal raphe nucleus in the rat: evidence for a minor dopaminergic component. *Exp. Brain Res.*, 73(1): 69–77.
- Kapur, S. and Remington, G. (1996) Serotonin–dopamine interaction and its relevance to schizophrenia. *Am. J. Psychiatry*, 153(4): 466–476.
- Katz, P.S. (1999) What are we talking about? modes of neuronal communication. In: Katz P.S. (Ed.), *Beyond Neurotransmission*. Oxford University Press, New York, pp. 1–28.
- Kebabian, J.W., Beaulieu, M. and Itoh, Y. (1984) Pharmacological and biochemical evidence for the existence of two categories of dopamine receptor. *Can. J. Neurol. Sci.*, 11(Suppl. 1): 114–117.
- Kelland, M.D., Freeman, A.S. and Chiodo, L.A. (1990) Serotonergic afferent regulation of the basic physiology and pharmacological responsiveness of nigrostriatal dopamine neurons. *J. Pharmacol. Exp. Ther.*, 253(2): 803–811.
- Kellendonk, C., Simposn, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H. and Kandel, E.R. (2006) Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron*, 49(4): 603–615.
- Kerenyi, L., Ticaurte, G.A., Schretlen, D.J., McCann, U., Varga, J., Matheus, W.B., Ravert, H.T., Dannals, R.F., Hilton, J., Wong, D.F. and Szabo, Z. (2003) Positron emission tomography of striatal serotonin transporters in Parkinson's disease. *Arch. Neurol.*, 60(9): 1223–1229.
- Kim, S.E., Choi, J.Y., Choe, Y.S., Choi, Y. and Lee, W.Y. (2003) Serotonin transporters in the midbrain of Parkinson's disease patients: a study with 123I-beta-CIT SPECT. *J. Nucl. Med.*, 44(6): 870–876.
- Kinney, J.W., Starosta, G. and Crawley, J.N. (2003) Central galanin administration blocks consolidation of spatial learning. *Neurobiol. Learn. Mem.*, 80(1): 42–54.
- Kirkby, R.J. and Polgar, S. (1974) Active avoidance in the laboratory rat following lesions of the dorsal or ventral caudate nucleus. *Physiol. Psychol.*, 2: 301–306.
- Kish, S.J. (2003) Biochemistry of Parkinson's disease: is a brain serotonergic deficiency a characteristic of idiopathic Parkinson's disease? *Adv. Neurol.*, 91(1): 39–49.
- Kish, S.J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L.J., Guttman, M. and Furukawa, Y. (2008) Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. *Brain*, 131(1): 120–131.
- Kiyatkin, E.A. and Rebec, G.V. (1999) Striatal neuronal activity and responsiveness to dopamine and glutamate after selective blockade of D1 and D2 dopamine receptors in freely moving rats. *J. Neurosci.*, 19(9): 3594–3609.
- Kostrzewa, R., Nowak, P., Kostrzewa, J., Kostrzewa, R. and Brus, R. (2005) Peculiarities of L-DOPA: treatment of Parkinson's disease. *Amino Acids*, 28(2): 157–164.
- Kuhn, W., Muller, T., Gerlach, M., Sofic, E., Fuchs, G., Heye, N., Prautsch, R. and Przuntek, H. (1996) Depression in Parkinson's disease: biogenic amines in CSF of "de novo" patients. *J. Neural Transm.*, 103(12): 1441–1445.
- Kupfermann, I. (1979) Modulatory actions of neurotransmitters. *Ann. Rev. Neurosci.*, 2: 447–465.
- Latgen, M., Elvander, E., Madjid, N. and Ogren, S.O. (2005) Analyses of the role of 5-HT1A receptors in spatial and

- aversive learning in the rat. *Neuropsychopharmacology*, 48(6): 830–852.
- Lee, E.H. and Geyer, M.A. (1984) Dopamine autoreceptor mediation of the effects of apomorphine on serotonin neurons. *Pharmacol. Biochem. Behav.*, 21(2): 301–311.
- Lehmann, O., Bertrand, F., Jeltsch, H., Morer, M., Lazarus, C., Will, B. and Cassel, J.C. (2002) 5,7-DHT-induced hippocampal 5-HT depletion attenuates behavioural deficits produced by 192 IgG-saporin lesions of septal cholinergic neurons in the rat. *Eur. J. Neurosci.*, 15(12): 1991–2006.
- Lemon, N. and Manahan-Vaughan, D. (2006) Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J. Neurosci.*, 26(29): 7723–7729.
- Lewis, S.J., Slabosz, A., Robbins, T.W., Barker, R.A. and Owen, A.M. (2005) Dopaminergic basis for deficits in working memory but not attentional set-shifting in Parkinson's disease. *Neuropsychologia*, 43(6): 823–832.
- Liao, R.M., Lai, W.S. and Lin, J.Y. (2002) The role of catecholamines in retention performance of a partially baited radial eight-arm maze for rats. *Chin. J. Physiol.*, 45(4): 177–185.
- Lucas, G., Di Matteo, V., De Deurwaerdère, P., Porras, G., Martín-Ruiz, R., Artigas, F., Esposito, E. and Spampinato, U. (2001) Neurochemical and electrophysiological evidence that 5-HT₄ receptors exert a state-dependent facilitatory control in vivo on nigrostriatal, but not mesoaccumbal, dopaminergic function. *Eur. J. Neurosci.*, 13(5): 889–898.
- MacDermott, A.B., Role, L.W. and Siegelbaum, S.A. (1999) Presynaptic ionotropic receptors and the control of transmitter release. *Annu. Rev. Neurosci.*, 22: 443–485.
- McDonald, R.J. and White, N.M. (1993) A triple dissociation of memory systems: hippocampus, amygdale, and dorsal striatum. *Behav. Neurosci.*, 107(1): 3–22.
- Maeda, T., Nagata, K., Yoshida, Y. and Kannary, K. (2005) Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered L-DOPA. *Brain Res.*, 1046(1–2): 230–233.
- Mamo, D., Graff, A., Mizrahi, R., Shammi, C.M., Romeyer, F. and Kapur, S. (2007) Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study. *Am. J. Psychiatry*, 164(9): 141–147.
- Matsuda, T., Sakaue, M., Ago, Y., Sakamoto, Y., Koyama, Y. and Baba, A. (2001) Functional alteration of brain dopaminergic system in isolated aggressive mice. *Nihon Shinkei Seishin Yakurigaku Zasshi.*, 21(3): 71–76.
- Matsumoto, M., Togashi, H., Mori, K., Ueno, K., Miyamoto, A. and Yoshioka, M. (1999) Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. *Eur. J. Pharmacol.*, 383(1): 39–48.
- Mayeux, R. (1990) The serotonin hypothesis for depression and Parkinson's disease. *Adv. Neurol.*, 53(1): 163–166.
- Mayeux, R., Stern, Y., Cote, L. and Williams, J.B. (1984) Altered serotonin metabolism in depressed patients with Parkinson's disease. *Neurology*, 34(5): 642–646.
- McDonald, R.J. and White, M.N. (1994) Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav. Neural. Biol.*, 61(3): 260–270.
- McDonald, R.J. and White, M.N. (1996) Hippocampal and non hippocampal contributions to place learning in rats. *Behav. Neurosci.*, 109(4): 579–593.
- Meltzer, H.Y., Li, Z., Kaneda, Y. and Ichikawa, J. (2003) Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 27(7): 1159–1172.
- Meneses, A. (2007) Stimulation of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}/2C, 5-HT₃, and 5-HT₄ receptors or 5-HT uptake inhibition: short- and long-term memory. *Behav. Brain Res.*, 184(1): 81–90.
- Meneses, A., Pérez-García, G., Liy-Salmeron, G., Flores-Galvez, D., Castillo, C. and Castillo, E. (2008) The effects of 5-HT(6) receptor agonist EMD and 5-HT(7) receptor agonist AS19 on memory formation. *Behav. Brain Res.*, doi:10.1016/j.bbr.2007.11.023.
- Micale, V., Leggio, G.M., Mazzola, C. and Drago, F.C. (2006) Cognitive effects of SL65.0155, a serotonin 5-HT₄ receptor partial agonist, in animal models of amnesia. *Brain Res.*, 1121(1): 207–215.
- Millan, M.J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)_{1A} receptors. *J. Pharmacol. Exp. Ther.*, 295(3): 853–861.
- Millan, M.J., Dekeyne, A. and Gobert, A. (1998) Serotonin (5-HT)_{2C} receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT release in the frontal cortex in vivo. *Neuropharmacology*, 37(7): 953–955.
- Miller, E.K. and Cohen, J.D. (2001) An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.*, 24: 167–202.
- Mirenowicz, J. and Schultz, W. (1994) Importance of unpredictability for reward responses in primate dopamine neurons. *J. Neurophysiol.*, 72(2): 1024–1027.
- Misane, I., Johansson, C. and Ögren, S.O. (1988) Analysis of the 5-HT_{1A} receptor involvement in passive avoidance in the rat. *Br. J. Pharmacol.*, 125(3): 499–509.
- Misane, I. and Ögren, S.O. (2000) Multiple 5-HT receptors in passive avoidance: comparative studies of p-chloroamphetamine and 8-OH-DPAT. *Neuropsychopharmacology*, 22(2): 168–190.
- Misane, I. and Ögren, S.O. (2002) Multiple 5-HT receptors in passive avoidance: comparative studies of p-chloroamphetamine and 8-OH-DPAT. *Neuropsychopharmacology*, 22(2): 168–190.
- Mishkin, M., Malamut, B. and Bachevalier, J. (1984) Memories and habits: two neuronal systems. In: McGrawHill J.L. and Weinberger N.M. (Eds.), *Neurobiology of Human Learning and Memory*. The Guilford press, New York, pp. 65–87.
- Mitchell, E.S. and Neumaier, J.F. (2005) 5-HT₆ receptors: a novel target for cognitive enhancement. *Pharmacol. Ther.*, 108(3): 320–333.
- Morrow, B.A., Elsworth, J.D., Rasmussen, A.M. and Roth, R.H. (1999) The role of mesoprefrontal dopamine neurons in

- the acquisition and expression of conditioned fear in the rat. *Neuroscience*, 92(2): 553–564.
- Müller, U., von Cramon, D.Y. and Pollmann, S. (1998) D1-versus D2-receptor modulation of visuospatial working memory in humans. *J. Neurosci.*, 18(7): 2720–2728.
- Murphy, D.L., Andrews, A.M., Wichems, C.H., Li, Q., Tohoda, M. and Greenberg, B. (1998) Brain serotonin neurotransmission: an overview and update with emphasis on serotonin system heterogeneity, multiple receptors, interactions with other neurotransmitter systems, and consequent implications for understanding the actions of serotonergic drugs. *J. Clin. Psychiatry*, 59(Suppl. 15): 4–12.
- Murtha, J.E.S. and Pappas, A.B. (1994) Neurochemical, histopathological and mnemonic effect of combined lesions of the medial septal and serotonin afferents to the hippocampus. *Brain Res.*, 651(1–2): 16–26.
- Naghdi, N. and Harooni, H.E. (2005) The effect of intrahippocampal injections of ritanserin (5HT_{2A/2C} antagonist) and granisetron (5HT₃ antagonist) on learning as assessed in the spatial version of the water maze. *Behav. Brain Res.*, 157(2): 205–210.
- Nakazato, T. (2005) Striatal dopamine release in the rat during a cued lever-press task for food reward and the development of changes over time measured using high-speed voltammetry. *Exp. Brain Res.*, 166(1): 137–146.
- Nayak, S.V., Rondé, P., Spier, A.D., Lummis, S.C. and Nichols, R.A. (2000) Nicotinic receptors co-localize with 5-HT₃ serotonin receptors on striatal nerve terminals. *Neuropharmacology*, 39(13): 2681–2690.
- Nedergaard, S., Bolam, J.P. and Greenfield, S.A. (1988) Facilitation of a dendritic calcium conductance by 5-hydroxytryptamine in the substantia nigra. *Nature*, 333(6169): 174–177.
- Normile, H.J., Jenden, D.J., Khun, D.M., Wolf, W.A. and Altman, H.J. (1990) Effects of combined serotonin depletion and lesions of the nucleus basalis magnocellularis on acquisition of a complex spatial discrimination task in the rat. *Brain Res.*, 563(1–2): 245–250.
- Ögren, S.O. (1985) Central serotonin neurons in avoidance learning: interactions with noradrenaline and dopamine neurons. *Pharmacol. Biochem. Behav.*, 23(1): 107–123.
- Ögren, S.O. (1986) Analysis of the avoidance learning deficit induced by the serotonin releasing compound *p*-chloroamphetamine. *Brain Res. Bull.*, 16(5): 645–660.
- Ögren, S.O. (2000) Multiple 5-HT receptors in passive avoidance: comparative studies of *p*-chloroamphetamine and 8-OH-DPAT. *Neuropsychopharmacology*, 22(2): 168–190.
- O'Hearn, E. and Molliver, M.E. (1984) Organization of raphe-cortical projections in rat: a quantitative retrograde study. *Brain Res. Bull.*, 13(6): 709–726.
- Olivera-Cortés, E., Brarajas-pérez, M., Morales Villagrán, A. and González-Burgos, I. (2001) Cerebral serotonin depletion induces egocentric learning improvement in developing rats. *Neurosci. Lett.*, 313(1–2): 29–32.
- Packard, M.G. and Knowlton, B.J. (2002) Learning and memory functions of the basal ganglia. *Annu. Rev. Neurosci.*, 25: 563–593.
- Packard, M.G. and McGaugh, J.L. (1994) Quinpirole and D-amphetamine administration post-training enhances memory on spatial and cued discriminations in a water maze. *Psychobiology*, 22: 54–60.
- Packard, M.G. and McGaugh, J.L. (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.*, 65(1): 65–72.
- Packard, M.G. and White, N.M. (1991) Dissociation of hippocampus and caudate nucleus memory systems by post-training intracerebral injection of dopamine agonist. *Behav. Neurosci.*, 105(2): 295–306.
- Palfreyman, M.G., Schmidt, C.J., Sorensen, S.M., Dudley, M.W., Kehne, J.H., Moser, P., Gittos, M.W. and Carr, A.A. (1993) Electrophysiological, biochemical and behavioral evidence for 5-HT₂ and 5-HT₃ mediated control of dopaminergic function. *Psychopharmacol. (Berl.)*, 112(Suppl. 1): S60–S67.
- Parent, A., Descarries, L. and Beaudet, A. (1981) Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [3H]5-hydroxytryptamine. *Neuroscience*, 6: 115–138.
- Pasquier, D.A., Kemper, T.L., Forbes, W.B. and Morgane, P.J. (1977) Dorsal raphe, substantia nigra and locus coeruleus: interconnections with each other and the neostriatum. *Brain Res. Bull.*, 2(5): 323–339.
- Paulus, W. and Jellinger, K. (1991) The neuropathological basis of different clinical subgroups of Parkinson's disease. *J. Neuropathol. Exp. Neurol.*, 50(6): 743–755.
- Pavese, N., Evans, A.H., Tay, Y.F., Hotton, G., Brooks, D.J., Lees, A.J. and Piccini, P. (2006) Clinical correlates of levodopa-induced dopamine release in Parkinson's disease: a PET study. *Neurology*, 67(9): 1612–1617.
- Pehek, E.A., McFarlane, H.G., Maguschak, K., Price, B. and Pluto, C.P. (2001) M100,907, a selective 5-HT_{2A} antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res.*, 888(1): 51–59.
- Pehek, E.A., Nocjar, C., Roth, B.L., Byrd, T.A. and Mabrouk, O.S. (2006) Evidence for the preferential involvement of 5-HT_{2A} serotonin receptors in stress- and drug-induced dopamine release in the rat medial prefrontal cortex. *Neuropsychopharmacology*, 31(2): 265–277.
- Pérez-vega, M.I., Feria-Velásco, A. and González-Burgos, I. (2000) Prefrontocortical serotonin depletion results in plastic changes of prefrontocortical pyramidal neurons, underlying greater efficiency of short-term memory. *Brain Res. Bull.*, 53(3): 291–300.
- Peyron, C., Petit, J.M., Rampon, C., Jouvet, M. and Luppi, P.H. (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience*, 82(2): 443–468.
- Pierucci, M., Di Matteo, V. and Espósito, E. (2004) Stimulation of serotonin_{2C} receptors blocks the hyperactivation of midbrain dopamine neurons induced by nicotine administration. *J. Pharmacol. Exp. Ther.*, 300(1): 109–118.
- Pizzagalli, D.A., Evins, A.E., Schetter, E.C., Frank, M.J., Pajtas, P.E., Santesso, D.L. and Culhane, M. (2008) Single

- dose of dopamine agonist impairs reinforcement learning in humans: behavioral evidence from a laboratory-based measure of reward responsiveness. *Psychopharmacol. (Berl.)*, 196(2): 221–232.
- Plasse, G., Meerkker, D.T.J., Lieben, C.K.J., Blokland, A. and Feenstra, M.G.P. (2007) Lack of evidence for reduced prefrontal cortical serotonin and dopamine efflux after acute tryptophan depletion. *Psychopharmacol. (Berl.)*, 195(3): 377–385.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1992) Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, 12(2): 440–453.
- Ponnusamy, R., Nissin, H.A. and Barad, M. (2005) Systemic blockade of D₂-like dopamine receptors facilitate extinction of conditioned fear in mice. *Learn. Mem.*, 12(4): 399–406.
- Porras, G., De Deurwaerdere, P., Moisson, D. and Spampinato, U. (2003) Conditional involvement of striatal serotonin₃ receptors in the control of in vivo dopamine outflow in the rat striatum. *Eur. J. Neurosci.*, 17(4): 771–781.
- Prado-Alcala, R.A., Grinberg, Z.J., Arditti, Z.L., Garcia, M.M., Prieto, H.G. and Brust-Carmona, H. (1975) Learning deficits produced by chronic and reversible lesions of the corpus striatum in rats. *Physiol. Behav.*, 15(3): 283–287.
- Prado-Alcalá, R.A., Ruiloba, M.I., Rubio, L., Solana-Figueroa, R., Medina, C., Salado-Castillo, R. and Quirarte, G.L. (2003a) Regional infusions of serotonin into the striatum and memory consolidation. *Synapse*, 47(3): 169–175.
- Prado-Alcalá, R.A., Solana-Figueroa, R., Galindo, L.E., Medina, A.C. and Quirarte, G.L. (2003b) Blockade of striatal 5-HT₂ receptors produces retrograde amnesia in rats. *Life Sci.*, 74(4): 481–488.
- Quednow, B.B., Jessen, F., Kuhn, K.U., Maier, W., Daum, I. and Wagner, M. (2006) Memory deficits in abstinent NDMA (ecstasy) users: neuropsychological evidence of frontal dysfunction. *J. Neuropsychopharmacol.*, 20(3): 373–384.
- Ramírez, M.J., Cenarruzabeitia, E., Lasheras, B. and Del Río, J. (1997) 5-HT₂ receptor regulation of acetylcholine release induced by dopaminergic stimulation in rat striatal slices. *Brain Res.*, 757(1): 17–23.
- Rampello, L., Chiechio, S., Rafaele, R.R., Vecchio, I. and Nicoletti, F. (2002) The SSRI citalopram improves bradykinesia in patients with Parkinson's disease treated with L-Dopa. *Clin. Neuropharmacol.*, 25(1): 21–24.
- Rebec, G.V., Christensen, J.R., Guerra, C. and Bardo, M.T. (1997) Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. *Brain Res.*, 776(1–2): 61–67.
- Reneman, L., Booij, J., Schmand, B., Van der Brink, W. and Gunning, B. (2000) Memory disturbances in "Ecstasy" users are correlated with an altered brain serotonin neurotransmission. *Psychopharmacology*, 148(3): 322–324.
- Richter-Levin, G. and Segal, M. (1989) Spatial performance is severely impaired in rats with combined reduction of serotonergic and cholinergic transmission. *Brain Res.*, 477(1–2): 404–407.
- Riedel, W.L., Klaannen, T., Deutz, N.E.P., Van Someten, A. and Van Praag, H.R. (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. *Psychopharmacol. (Berl.)*, 141(4): 362–369.
- Robbins, T.W. and Roberts, A.C. (2007) Differential regulation of fronto-executive function by the monoamines and acetylcholine. *Cereb. Cortex. Suppl.*, 1: i151–i160.
- Robinson, D.L., Phillips, P.E., Budygin, E.A., Trafton, B.J., Garris, P.A. and Wightman, R.M. (2001) Sub-second changes in accumbal dopamine during sexual behavior in male rats. *Neuroreport*, 12(11): 2549–2552.
- Roitman, M.F., Stuber, G.D., Phillips, P.E., Wightman, R.M. and Carelli, R.M. (2004) Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci.*, 24(6): 1265–1271.
- Rondé, P. and Nichols, R.A. (1998) High calcium permeability of serotonin 5-HT₃ receptors on presynaptic nerve terminals from rat striatum. *J. Neurochem.*, 70(3): 1094–1103.
- Roth, B.L., Craigo, S.C., Choudhary, M.S., Uluer, A., Monsma, F.J., Jr., Shen, Y., Meltzer, H.Y. and Sibley, D.R. (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.*, 268(3): 1403–1410.
- Ruotsalainen, S., McDonald, E., Koivisto, E., Stefanski, R., Haapalinna, A., Riekkinen, P., Jr. and Sirviö, J. (1998) 5-HT_{1A} receptor agonist (8-OH-DPAT) and 5-HT₂ receptor agonist (DOI) disrupt the non-cognitive performance of rats in a working memory task. *J. Psychopharmacol.*, 12(2): 177–185.
- Sakai, K., Salvert, D., Touret, M. and Jouvet, M. (1977) Afferent connections of the nucleus raphe dorsalis in the cat as visualized by the horseradish peroxidase technique. *Brain Res.*, 137(1): 11–35.
- Salomone, J.D., Cousins, M.S. and Snyder, B.J. (1997) Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. *Neurosci. Biobehav. Rev.*, 21(3): 341–359.
- Sawaguchi, T. and Goldman-Rakic, P.S. (1991) D₁ dopamine receptors in prefrontal cortex: involvement in working memory. *Science*, 251(4996): 947–950.
- Scatton, B., Javoy-Agid, F., Rouquier, L., Dubois, B. and Agid, Y. (1983) Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. *Brain Res.*, 275(2): 321–328.
- Schmitt, J.A., Jorissen, B.L., Sobczak, S., van Bostel, M.P., Hogervorst, E., Deutz, N.E. and Riedel, W.J. (2000) Tryptophan depletion impairs memory consolidation but improves focussed attention in healthy young volunteers. *J. Psychopharmacol.*, 14(1): 21–29.
- Scholes, K.E., Harrison, B.J., O'Neurill, B.V., Leung, S., Croft, R.J., Pipingas, A., Phan, K.L. and Nathan, P.J. (2007) Acute serotonin and dopamine depletion improves attentional control: findings from the stroop task. *Neuropsychopharmacology*, 32(7): 1600–1610.
- Scholtissen, B., Verhey, F.R.J., Adam, J.J., Prickaerts, J. and Leentjens, A.F. (2006) Effects of acute tryptophan depletion

- on cognition, memory and motor performance in Parkinson's disease. *J. Neurol. Sci.*, 248(1–2): 259–265.
- Schultz, W. (1997) Dopamine neurons and their role in reward mechanisms. *Curr. Opin. Neurobiol.*, 7(2): 191–197.
- Schultz, W. (1998) Predictable reward signal of dopamine neurons. *J. Neurophysiol.*, 80(1): 1–27.
- Schultz, W., Dayan, P. and Montague, P.R. (1997) A neural substrate of prediction and reward. *Science*, 275(5306): 1593–1599.
- Seamans, J.K., Durstewitz, D., Christie, B.R., Stevens, C.F. and Sejnowski, T.J. (2001a) Dopamine D1/D5 receptor modulation of excitatory synaptic inputs to layer V prefrontal cortex neurons. *Proc. Natl. Acad. Sci. U.S.A.*, 98(1): 301–306.
- Seamans, J.K., Gorelova, N., Durstewitz, D. and Yang, C.R. (2001b) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J. Neurosci.*, 21(10): 3628–3638.
- Seamans, J.K. and Yang, C.R. (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog. Neurobiol.*, 74(1): 1–58.
- Sesack, S.R. and Carr, D.B. (2002) Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. *Physiol. Behav.*, 77(4–5): 513–517.
- Sesack, S.R. and Pickel, V.M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.*, 320(2): 145–160.
- Shapovalova, K.B. and Kamkina, Y.V. (2008) Motor and cognitive functions of the neostriatum during bilateral blockade of its dopamine receptors. *Neurosci. Behav. Physiol.*, 38(1): 71–79.
- Simon, H., Scatton, B. and Moal, M.L. (1980) Dopaminergic A10 neurons are involved in cognitive functions. *Neurosci. Lett.*, 15(2–3): 319–324.
- Sleight, A.J., Consolo, S., Martin, J.R., Boes, M., Boess, F.G., Bentley, J.C. and Bourson, A. (1999) 5-HT₆ receptors: functional correlates and potential therapeutic indications. *Behav. Pharmacol.*, 10: p. S86.
- Smith, A.D., Smith, D.L., Zigmond, M.J., Amalric, M. and Koob, G.F. (2000) Differential effects of dopamine receptors subtype blockade on performance of rats in a reaction-time paradigm. *Psychopharmacology*, 148(4): 355–360.
- Soghomonian, J.J., Descarries, L. and Watkins, K.C. (1989) Serotonin innervation in adult rat neostriatum II ultrastructural features: a radioautographic and immunocytochemical study. *Brain Res.*, 481(1): 67–86.
- Solana-Figueroa, R., Salado-Castillo, R., Galindo, L.E., Quitarte, G.L. and Prado-Alcalá, R.A. (2002) Effects of pretraining with intrastriatal administration of *p*-chloroamphetamine on inhibitory avoidance. *Neurobiol. Learn. Mem.*, 78(1): 178–185.
- Schreiber, R., Vivian, J., Hedley, L., Szczepanski, K., Secchi, R.L., Zuzow, M., van Laarhoven, S., Moreau, J.-L., Martin, J.R. and Blokland, A. (2007) Effects of the novel 5-HT₆ receptor antagonist RO4368554 in rat model for cognition and sensorimotor gating. *Eur. Neuropsychopharmacol.*, 17: 277–288.
- Staübli, U. and Xu, B. (1995) Effects of 5-HT₃ receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. *J. Neurosci.*, 15(3, pt 2): 2445–2452.
- Stone, J.M. and Pilowsky, L.S. (2007) Novel targets for drugs in schizophrenia. *CNS Neurol. Disord. Drug Targets*, 6(4): 265–272.
- Stoof, J.C. and Keibian, J.W. (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature*, 294(5839): 366–368.
- Stuchlik, A. (2007) Further study of the effects of dopaminergic D1 drugs on place avoidance behavior using pretraining: some negative evidence. *Behav. Brain Res.*, 178(1): 47–52.
- Stuchlik, A., Rehakova, L., Telensky, P. and Vales, K. (2007) Morris water maze learning in Long-Evans rats is differentially affected by blockade of D1-like and D2-like dopamine receptors. *Neurosci. Lett.*, 422(3): 169–174.
- Stuchlik, A., Rezacova, L., Vales, K., Bubenikova, V. and Kubuk, S. (2004) Application of a novel allothetic place avoidance task (AAAPA) in testing a pharmacological model of psychosis in rats: comparison with the Morris water maze. *Neurosci. Lett.*, 366(2): 162–166.
- Stuchlik, A. and Vales, K. (2006) Effect of dopamine D1 receptor antagonist SCH23390 and D1 agonist A77636 on active allothetic place avoidance, a spatial cognition task. *Behav. Brain Res.*, 172(2): 250–255.
- Sutton, M.A., Rolfe, N.G. and Beninger, R.J. (2001) Biphasic effects of 7-OH-DPAT on the acquisition of responding for conditioned reward in rats. *Pharmacol. Biochem. Behav.*, 69(1–2): 195–200.
- Suzuki, T., Miura, M., Nishimura, K. and Aosaki, T. (2001) Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *J. Neurosci.*, 21(17): 6492–6501.
- Tanila, H., Björklund, M. and Riekkinen, P., Jr. (1998) Cognitive changes in mice following moderate MPTP exposure. *Brain Res. Bull.*, 45(6): 577–582.
- Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T. and Matsunaga, M. (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport*, 10(3): 631–634.
- Tinaz, S., Schendan, H.E., Schon, K. and Stern, C.E. (2006) Evidence for the importance of basal ganglia output nuclei in semantic event sequencing: an fMRI study. *Brain Res.*, 1067(1): 239–249.
- Trantham-Davidson, H., Kröner, S. and Seamans, J.K. (2008) Dopamine modulation of prefrontal cortex interneurons occurs independently of DARPP-32. *Cereb. Cortex.*, 0: bhm133v1.
- Trantham-Davidson, H., Neely, L.C., Lavin, A. and Seamans, J.K. (2004) Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. *J. Neurosci.*, 24(47): 10652–10659.

- Trimmer, B.A. (1999) The messenger is not the message; or is it? In: Katz P.S. (Ed.), *Beyond Neurotransmission*. Oxford University Press, New York, pp. 29–82.
- Truffinet, P., Tamminga, C., Fabre, L.F., Meltz, H.Y., Riviere, M.-E. and Papillon-Downey, C. (1999) Placebo-controlled study of the D4/5-HT_{2A} antagonist fananserin in the treatment of schizophrenia. *Am. J. Psychiatry*, 156(3): 418–425.
- Tseng, K.Y. and O'Donnell, P. (2004) Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. *J. Neurosci.*, 44(5): 1526–1539.
- Ugedo, L., Grenhoff, J. and Svensson, T.H. (1989) Ritanerlin, 5-HT₂ receptor antagonist, activate midbrain dopamine neurons by blocking serotonin inhibition. *Psychopharmacol. (Berl.)*, 98(1): 45–50.
- Vales, K. and Struchlik, A. (2005) A central muscarinic blockade interferes with learning and retrieval of the active allothetic place avoidance task despite spatial pretraining. *Behav. Brain Res.*, 161: 238–244.
- Vertes, R.P. (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse*, 51(1): 32–58.
- Ward, J., Hall, K. and Haslam, C. (2006) Patterns of memory dysfunction in current and 2-year abstinent NDMA users. *J. Clin. Exp. Neuropsychol.*, 28(3): 306–324.
- Ward, B.O., Wilkinson, L.S., Robbins, T.W. and Everitt, B.J. (1999) Forebrain serotonin depletion facilitates the acquisition and performance of a conditional visual discrimination task in rats. *Behav. Brain Res.*, 100(1–2): 51–65.
- Watanabe, K. and Kimura, M. (1998) Dopamine receptor-mediated mechanisms involved in the expression of learned activity of primate striatal neurons. *J. Neurophysiol.*, 79(5): 2568–2580.
- Wenk, G., Hughey, D., Boundy, V., Kim, A., Walker, L. and Olton, D. (1987) Neurotransmitters and memory: role of cholinergic, serotonergic, and noradrenergic systems. *Behav. Neurosci.*, 101(3): 325–332.
- Werkman, T.R., Glennon, J.C., Wadman, W.J. and McCreary, A.C. (2006) Dopamine receptor pharmacology: interactions with serotonin receptors and significance for the aetiology and treatment of schizophrenia. *CNS Neurol. Disord. Drug Targ.*, 5(1): 3–23.
- West, A.R. and Grace, A.A. (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. *J. Neurosci.*, 22(1): 294–304.
- White, N.M., Packard, M.G. and Seamans, J. (1993) Memory enhancement by post-training peripheral administration of low doses of dopamine agonists: possible autoreceptor effect. *Behav. Neural Biol.*, 59(3): 230–241.
- White, N.M. and Salinas, J.A. (2003) Mnemonic functions of dorsal striatum and hippocampus in aversive conditioning. *Behav. Brain Res.*, 142(1–2): 99–107.
- Willins, D.L. and Meltzer, H.Y. (1998) Serotonin 5-HT_{2C} agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. *Brain Res.*, 781(1–2): 291–299.
- Wightman, R.M. and Robinson, D.L. (2002) Transient changes in mesolimbic dopamine and their association with 'reward'. *J. Neurochem.*, 82(4): 721–735.
- Wilkerson, A. and Levin, E.D. (1999) Ventral hippocampal dopamine D1 and D2 systems and spatial working memory in rats. *Neuroscience*, 89(3): 743–749.
- Wilson, Ch.J. (1998) Basal ganglia. In Shepherd, G. M (Ed.), *The Synaptic Organization of the Brain*, Fourth edn. Chap. 9, pp. 329–375.
- Winocur, G. (1974) Functional dissociation within the caudate nucleus of rats. *J. Comp. Physiol. Psychol.*, 86(3): 432–439.
- Winterer, G. and Weinberger, D.R. (2004) Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci.*, 27(11): 683–690.
- Wolf, M.E., Deutch, A. Y., and Roth, R.H. (1987) Pharmacology of central dopaminergic neurons. In F.A. Henn & L.E. DeLisi (Eds.), *Handbook of Neurochemistry and Neuropharmacology of Schizophrenia*, New York: Elsevier Science Publishers BV. Vol. 2., Chap. 4, pp. 101–147.
- Wolterink, G., Phillips, G., Cador, M., Donselaar-Wolterink, I., Robbins, T.W. and Everitt, B.J. (1993) Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. *Psychopharmacol. (Berl.)*, 110(3): 355–364.
- Wood, M.D., Reavill, C., Trail, B., Wilson, A., Stean, T., Kennett, G.A., Lightowler, S., Blackburn, T.P., Thomas, D., Gager, T.L., Riley, G., Holland, V., Bromidge, S.M., Forbes, I.T. and Middlemiss, D.N. (2001) SB-243213, a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. *Neuropharmacology*, 41(2): 186–199.
- Yin, H.H., Knowlton, B.J. and Balleine, B.W. (2004) Lesions of the dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur. J. Neurosci.*, 19(1): 181–189.
- Yu, L. and Liao, P.C. (2000) Estrogen and progesterone distinctively modulate methamphetamine-induced dopamine and serotonin depletions in C57BL/6J mice. *J. Neural. Transm.*, 107(10): 1139–1147.
- Zahrt, J., Taylor, J.R., Mathew, R.G. and Arnsten, A.F. (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J. Neurosci.*, 17(21): 8528–8535.
- Zakzanis, K.K., Campbell, Z. and Jovanovski, D. (2007) The neuropsychology of ecstasy (NDMA) use: a review. *Hum. Psychopharmacol.*, 22(7): 427–435.
- Zheng, P., Zhang, X.X., Bunney, B.S. and Shi, W.X. (1999) Opposite modulation of cortical *N*-methyl-D-aspartate receptor-mediated responses by low and high concentrations of dopamine. *Neuroscience*, 91(2): 527–535.
- Zhou, F.M. and Hablitz, J.J. (1999) Activation of serotonin receptors modulates synaptic transmission in rat cerebral cortex. *J. Neurophysiol.*, 82(6): 2989–2999.

CHAPTER 28

Serotonin/dopamine interaction in memory formation

Ignacio González-Burgos^{1,2,*} and Alfredo Feria-Velasco²

¹Laboratorio de Psicobiología, División de Neurociencias, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jal., México

²Laboratorio de Neurobiología Celular, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara. Guadalajara, Jal., México

Abstract: Both serotonin (5-HT) and dopamine (DA) neurotransmitters play a key role in modulating synaptic transmission in the central nervous system. Such 5-HT- and DA-mediated modulatory activity has been shown to influence a wide variety of cerebral functions, both of an instrumental and cognitive nature. Some brain regions strongly involved in cognition such as the prefrontal cortex, hippocampal formation and *corpus striatum*, are densely innervated by serotonergic and dopaminergic afferents proceeding from the raphe complex and the mesocorticolimbic or nigrostriatal systems, respectively. Learning and memory are strongly modulated by 5-HT and DA neurotransmitter activity, and in some cases they interact interdependently to sustain the psychobiological organization of these cognitive processes. Learning and memory, at least in part, depend on short- or long-lasting synaptic modifications, mainly occurring at dendritic spines. Indeed, the modulatory influence of 5-HT and DA at the synaptic level may affect the codification of mnemonic information on such spines. In fact, several experimental models of neurotransmitter activity have identified a close association between a 5-HT–DA imbalance and cytoarchitectonic changes underlying learning and memory impairment.

Keywords: adaptive; behavior; serotonin; dopamine; learning; memory; dendritic spines

Introduction

Our understanding of the neurobiological phenomena underlying the organization of behaviour has required experimental studies capable of discriminating the participation of the variables involved in these processes. As a result, it is clear that the neurotransmitter activity of different chemical substances is capable of producing a firing pattern

in nerve cells. This implies that when confronted with diverse environmental situations, the information that the brain processes through its balanced biochemical activity is incorporated into reference schemes that reflect the harmonious relationship that individuals maintain with their physical and even psychological environment. On the other hand, the imbalances that might occur under atypical conditions could well lead to discordant individual–environment interactions with respect to the needs of the former, which would provoke behavioural disruptions that will impair the individual's capacity to adapt to the situation (Fig. 1).

*Corresponding author. Tel.: +52 333 6683000–31950;
Fax: +52 333 6181756; E-mail: igonbur@hotmail.com

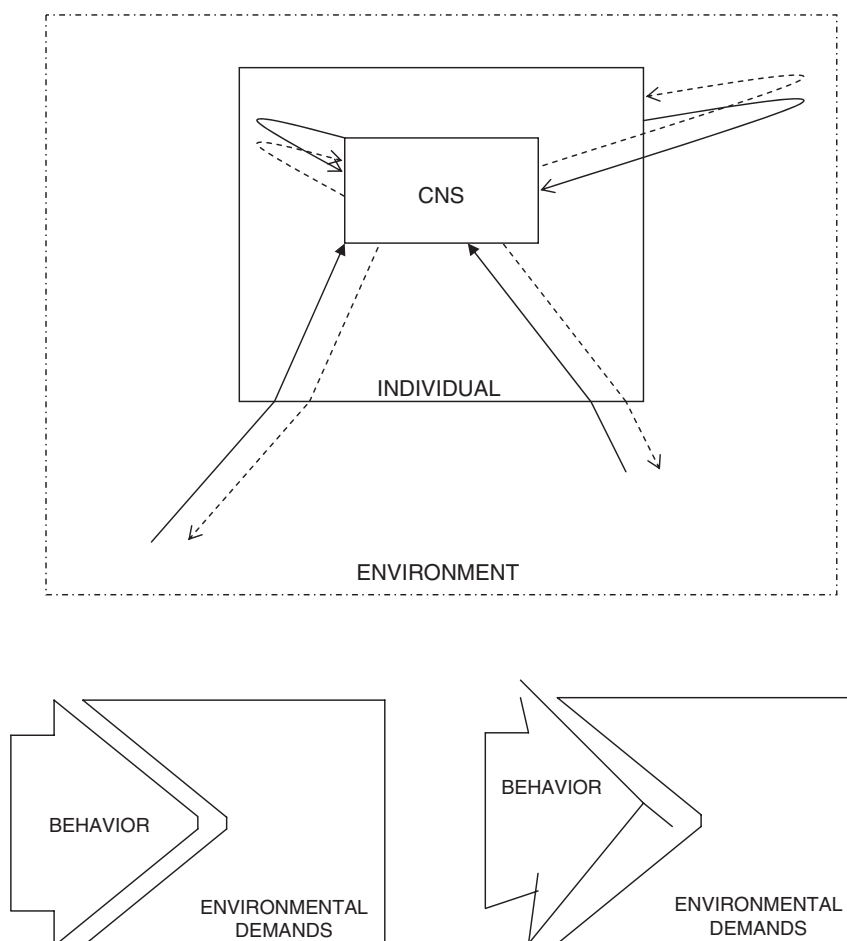


Fig. 1. Schematic representation of the interactive relationships between an individual and his environment. This bidirectional interaction (upper panel) is characterized by the sensorial (straight lines) and psychological (curve lines) afferent stimulation (solid arrows) to which an individual is permanently exposed; such afferent information is analysed in the brain (CNS) leading in turn to structuring behavioural responses (dotted arrows) putatively correspondent to the environmental demands. If such behavioural responses are in agreement with these, then harmonic adaptation is facilitated in healthy subjects (lower panel; left); on the contrary, aberrant behaviour could be closely related with psychopathological disorders (lower panel; right).

Over and above the observational criteria, the concept of 'behaviour' could be conceptualized as the result of an individual's interaction with his environment. From an operational point of view, the information the subject 'extracts' from such an interaction is incorporated into abstract representations in the brain. Such representations are contrasted both with prior reference schemes, such as genetically determined response patterns that finally permit reflex, invariable, conditioned actions to be taken or by taking decisions to

resolve the problems that such an interaction poses. In this context, the brain activity translates into motor actions, which carries an implicit or explicit message that acquires a higher character during the process of verbal communication in human beings.

Motor activity (or its absence) results from the processing of the information obtained during this interaction between the individual and his environment, and is interpreted as 'behaviour'. Thus, behaviour would be the series of messages emerging

from the nervous system in response, not only to certain stimuli but also to the information acquired from the environment. This information is decoded, ordered and recoded by virtue of the activation of neuronal systems that produce an action, be it a reflex, or a fixed pattern conditioned by prior experience, or by reference schemes derived from specific situations that require a decision to be taken in response to a specific problem.

Within this framework, brain activity acquires a preponderant character in the interpretation of behaviour, for which the experimental study of the underlying psychoneural processes and the neurobiological phenomena that they sustain, are critical to understand their expression, both under normal and pathological circumstances.

Whatever the psychobiological resources with which an individual confronts the needs constantly presented by their interaction with their environment, the fundamental objective of their activity is to achieve a satisfactory adaptation to this. To achieve this process of adaptation satisfactorily, intrinsic instrumental and cognitive capacities participate in a relevant fashion, among which learning and memory stand out.

The normal psychoneural expression of learning and memory capacity is regulated by the balanced activity of the diverse brain neurotransmitter systems. The metabolic imbalance of such systems — be it genetic or environmental in origin — could produce behavioural disorders that in the worst case, would impede a harmonious relationship in accordance with the demands of the surroundings. Thus, the individuals that develop disorders in the cognitive sphere related with alterations in the neurochemical balance of some neurotransmitter systems are incapable of harmoniously interacting with their social and psychological surroundings.

Two of the neurotransmitters closely related with cognitive function are 5-hydroxytryptamine (5-HT; serotonin) and dopamine (DA). As well as their neurotransmitter activity, these two biogenic amines possess neuromodulatory activity. They can thus exert a direct or indirect influence on the excitability of neurons that they stimulate.

The neurons that liberate 5-HT and DA are located in specific nervous centres. The axon terminals of serotonergic and dopaminergic

neurons are localized in different brain regions in which they exert their neurotransmitter and/or neuromodulatory effects. The postsynaptic bioelectric effect after their release depends on the transduction system of these chemical signals, made up of the receptor molecules for the corresponding neurotransmitter and where appropriate, by those that modulate the neuronal response to other chemical stimuli through their secondary activity. Thus, the psychoneural effect that they exert will depend on the brain region in question. Both 5-HT and DA are released in brain regions such as the cerebral cortex, the hippocampus and the corpus striatum. These three brain regions are involved in the organization of diverse cognitive processes, most noticeably, learning and memory.

Serotonergic and dopaminergic nerve terminals may have an excitatory, inhibitory or modulatory effect depending on the chemical receptor located in the postsynapse. Furthermore, the neurotransmission mediated by 5-HT could be affected to some extent by DA and vice versa. The terminals that liberate 5-HT do so both in the synaptic cleft of specific contacts or as free terminals, whilst DA acts exclusively onto receptors located in the postsynaptic membrane.

Learning and memory processes are partially regulated by the neurotransmitter and neuromodulatory activity of 5-HT and DA. Such regulation is intrinsically related to the expression of morphophysiological phenomena of synaptic plasticity that occur at dendritic spines of the neurons that possess them. Thus, the alteration of serotonergic activity can affect the excitability of certain brain regions by virtue of modifications in the cytoarchitectonic pattern of principal neurons present therein. Moreover, dopaminergic terminals may be located in the neck of the dendritic spines on principal projection neurons, both in the prefrontal cerebral cortex as well as in the striatum, while glutamatergic terminals are situated in the head of these same spines. Therefore, a modulatory effect of DA on the excitatory information afferent to these neurons has been proposed. It then follows that the modifications in the neuronal cytoarchitectonic pattern induced by changes in the 5-HT and DA neurotransmitter activity can affect the organization of the information related to learning and memory.

An overview of learning and memory processes

Learning and memory are two cognitive processes related to the processing of the information that permits an individual to successfully adapt to his environment.

Learning can be conceptualized as the capacity to acquire information from internal and/or external environmental stimulation, which is potentially capable of altering behavioural responses. On the other hand, memory can be conceptualized as the process by which the information acquired during learning is stored and later retrieved (Sweatt, 2003).

The 'formation' of the memory involves three basic stages: (1) the *acquisition* of the information; (2) the *consolidation* of the information; and (3) the *storage* of the information. The information obtained from the environment is incorporated into a type of elementary cerebral 'buffer' through the sensory organs until it reaches primary cortical regions (visual, auditive, etc.) (Başar, 2004). This information is then structured into generally referenced schematic representations to finally create a species of information archive through mnemonic traces that are stable for a certain period of time. This implies that learning cannot be considered — or studied — as an isolated process. In fact, learning becomes evident after the manifestation of motor actions that reflect the recall of previously learned information. Thus, learning and memory are two closely related cognitive processes that if they are to be understood, will require their correlative study.

The adequate *storage* of information implies its prior *encoding* (Başar, 2004), that is, the 'accommodation' of the *items* or elements in such a manner that the corresponding representations are in accordance with the environmental conditions that generated them. This facilitates the appropriate retrieval of such information and under normal conditions the structuring of the behavioural response can be achieved in accordance with the demands presented by the external or internal sensory environmental stimulation, or even by evoking pre-existing psychic representations. In summary, the joint process of learning and memory is made up of the capacity to acquire,

encode, store, maintain and recall the information coming from the surroundings.

Independent of the psychoneural processing of information, memory has been classified in various categories or *systems* (Squire, 1992) so that it can be understood and studied.

In terms of the type of information, the processes of learning and memory have been classified as *declarative* or *explicit* memory, also called *conscious learning*, and *non-declarative* or *implicit* memory, also called *unconscious learning* (Sweatt, 2003).

Declarative memory implies unconscious storage but conscious recall of the information. It could refer to events (*episodic memory*), or to facts or isolated data (*semantic memory*). Likewise, it includes conscious associative conditioning and spatial learning. Since information recall in the declarative memory is conscious, it follows that cortical areas — including the hippocampus — are mainly involved in its organization.

The storage of information corresponding to implicit memory is also unconscious, but its recall can be either conscious or unconscious. Consciously evoking this information is implicated in tasks of *operant behaviour*. The neural pathways implicated in operant conditioning include the prefrontal cortex, the nucleus accumbens and the amygdala. Alternatively, unconsciously evoking memories is included in non-associative learning tasks such as *habituation*, *dishabituation* and *sensitization* (in which the participation of reflex pathways stands out), as well as tasks of associative learning such as *simple classical* conditioning. Particularly in this type of conditioning some thalamic nuclei are related to the specific sensory inputs; the amygdala is directly involved in the emotive and motivational content whilst the motor aspects are strongly influenced by the cerebellar activity. Similarly, in unconsciously evoking implicit memories, including *procedural memory* that contains the capacity to acquire habits and motor skills the repetition of which tends to increase the dexterity of their execution. The activity of the corpus striatum is relevant to the organization of this procedural memory, as is that of the cerebellum, the thalamus and the motor cortex. Further, *phyletic memory* refers to those patterns of perceptual references inherited during evolution

and that are evoked by specific stimuli or by the 'need to act'. *Perceptual memory* is another form of implicit memory that is evoked unconsciously and refers to the neocortical representation of events, objects, people, animals, facts, names and concepts, ranging from elementary sensations to the formation of abstract concepts.

It has been suggested that only the declarative or explicit memory can have a temporal dimension (Squire, 1992). In this context, and in accordance with the time over which the information is maintained until it is later recalled, this memory has been classified as *long-term* and *short-term memory* (Baddeley, 2000). Comparatively, long-term memory is more stable and less labile and the information can be maintained for hours, days or years. As a specialization of cognitive short-term memory, the *working memory* (or *active short-term memory*) refers to the actualization in the short-term of items of information that are specifically necessary during the execution of a certain task. The psychoneural dynamic of this type of short-term memory is sustained by the conformation of dynamic networks between prefrontocortical neurons denominated 'memory fields'; these are alternating, temporally reverberant and remain active and stable until the behavioural action is executed (Williams and Goldman-Rakic, 1995). Both evoking memories and executing motor actions leading to the execution of tasks related to the working memory (also called operant) requires the fully conscious processing of the information (Sweatt, 2003).

It is therefore evident that the participation of the cerebral cortex, hippocampus, striatum, amygdala and cerebellum are particularly important in the functional organization of diverse components of learning and memory.

5-HT in learning and memory

5-HT is synthesized from tryptophan obtained through the diet. The experimental manipulation of the availability of this essential amino acid has been used as an experimental paradigm to study the effect that 5-HT exerts over different psychoneural processes such as learning and memory.

It has been shown that the generalized depletion of 5-HT in the brain produces an impairment of short-term memory but not of long-term memory (Hritcu et al., 2007). However, there is also evidence that the availability of tryptophan in the brain affects both types of memory. It has also been reported that the restriction of tryptophan produces impairment in the formation of the long-term memory and its consolidation (Schmitt et al., 2000), which would agree with later findings that 5-HT affects this type of memory by affecting the information encoding phase rather than recall (van der Veen et al., 2006). Alternatively, the activity of 5-HT is related to the acquisition, retention and recuperation of the information associated with short-term memory. This effect is more pronounced in long-term memory, suggesting that both types of memory are processed independently (Shirahata et al., 2006).

In repetitive trial assays of short-term memory, greater behavioural efficiency was achieved under conditions of chronic tryptophan depletion (González-Burgos et al., 1998), which was confirmed in a similar behavioural paradigm after producing a chemical lesion of the serotonergic raphe-prefrontal pathway (Pérez-Vega et al., 2000). These findings could be related to the effects that 5-HT exerts on behavioural flexibility. Indeed, it has been reported that the depletion of prefrontal 5-HT produces enduring behaviours, without affecting the capacity to retain or discriminate those previously learned (Clarke et al., 2004). In accordance with this, tryptophan restriction improves the short-term active memory (working memory) (Riedel et al., 2003), which is dependent on prefrontal cortical activity (Fuster, 1997). This would be supported by the activation of 5-HT_{2A} receptors located in prefrontal pyramidal neurons that are known to facilitate the spatial working memory performance (Williams et al., 2002). Similarly, a higher density of 5-HT_{2A} receptors has been observed after prefrontal 5-HT depletion (sent to publication), as well as an enhancement of multiunitary activity of prefrontal neurons (sent to publication). These would be strongly associated with a more efficient short-term memory performance along with cytoarchitectural changes in prefrontal pyramidal

neurons pointing to a greater synaptic efficiency (Pérez-Vega et al., 2000; Feria-Velasco et al., 2002).

Together, these findings are in accordance with the increase in the focused attention capacity observed under conditions of tryptophan restriction (Schmitt et al., 2000), which is an indispensable prerequisite for mnemonic information processing. Also, it has been proposed that the regulation of the attention capacity could result from the inhibitory effect of 5-HT on other neurotransmitters involved in this process, such as norepinephrine and acetylcholine (Bell et al., 2001). Such inhibition could facilitate the acquisition of information within short-term memory (Masaki et al., 2006) until abnormally high values are reached in conditions of tryptophan depletion.

Like any other neurotransmitter, the activity of 5-HT is mediated by specific receptors. Accordingly, the synaptic and physiological effects of serotonergic synapses depend on the type of receptor that is stimulated in the synapse. Likewise, these synapses can also be influenced by possible interactions between these serotonergic terminals and other neurotransmitter systems such as the cholinergic system in the hippocampus, cortex and striatum, where both systems cooperate in the regulation of cognitive functions (Cassel and Jeltsch, 1995).

The 5HT receptors have been grouped into seven classes, each of which is subdivided into subtypes (Barnes and Sharp, 1999; Meneses, 1999) (Table 1). All these receptors, except the 1E, 1F, 4C and 4D, have been located in areas related with learning and memory, such as the hippocampus, amygdala and cerebral cortex (Meltzer et al., 1998).

The 5HT_{1A} receptors are closely associated with learning and memory (Meneses and Perez-Garcia, 2007). Despite being the most widely studied 5-HT receptors, experimental studies with agonists and antagonists have produced unclear results. In general, they do not affect or interfere with the acquisition, consolidation and retention of learning and memory in different tests (see Meneses, 1999, for review). The difficulty of interpreting the effects of their stimulation lie in the variation in the behavioural tests used, the duration of the

Table 1. Serotonin receptors

Family	Subtype
5-HT ₁	1A
	1B
	1D
	1E
	1F
5-HT ₂	2A
	2B
	2C
5-HT ₃	3A
	3B
5-HT ₄	4A
	4B
	4C
	4D
5-HT ₅	5A
	5B
5-HT ₆	6
5-HT ₇	7B
	7C
	7D

training, the brain areas involved and their localization pre- or postsynaptically (Meneses and Perez-Garcia, 2007). Such problems combine with the strong serotonergic influence that exists on cholinergic, GABAergic and glutamatergic transmission mediated by 1A receptors in the raphe complex, amygdala, septum, hippocampus and cerebral cortex, in relation to cognitive processes (Meneses, 1998). Thus, it is known that the blockade of 5HT_{1A} receptors produces a pro-cognitive effect by facilitating glutamatergic neurotransmission (Schiapparelli et al., 2006), which is in accordance with findings in humans showing that their activation has negative effects on explicit verbal memory (Yasuno, 2004).

The 1B, 1D, 2A, 2B and 2C receptors have been most specifically related to the acquisition and consolidation of learning (Meneses, 1999). It has been reported that the blockade of 5HT₂ receptors produces retrograde amnesia in rats, affecting the consolidation of memory (Prado-Alcalá et al., 2003a).

Of all the 5-HT receptors, the type 3 receptors are the only ones coupled to ion channels (Peters et al., 1992); they also modulate the activity of the cholinergic and glutamatergic systems in the

amygdala, hippocampus and entorhinal cortex (Meneses, 1998). These receptors are located in the soma, axon and/or nerve terminals of GABAergic interneurons (Zifa and Fillion, 1992), and it has been reported that they participate in the synaptic organization of information related to learning and memory (Staubli and Xu, 1995). Indeed, the application of antagonists to these receptors in the amygdala provokes an improvement in learning (Costall and Naylor, 1997).

The 5-HT₄ receptors are located in the habenula, hippocampus and the amygdala, and they mediate the slow excitatory and the long-lasting response in the hippocampus (Eglen et al., 1995).

The 5HT₆ receptor is particularly abundant in the olfactory bulb, striatum, nucleus accumbens, cerebral cortex and some sub-fields of the hippocampus (Gerard et al., 1996). The blockade of the 5HT₆ receptors with antagonists in the rat prefrontal cortex and hippocampus increases excitatory neurotransmission, which suggests their localization in interneurons or extrinsic GABAergic terminals (Dawson et al., 2001).

The antagonism of the 1A, 2A, 2B, 4 or 6 receptors improves only long-term memory, while antagonism of 1B receptors improves both long- and short-term memory. This suggests that both types of memory appear to function in parallel using the same signalling cascades, whilst on other occasions they are functionally associated in series (Meneses, 2007). It has been shown that serial activity of short- to long-term memory is favoured by the activity of 5HT_{1B} receptors, whilst the 1A, 2A, 2B/2C, 4 and 6 receptors are involved in their activity in parallel (Meneses, 2007).

Both the dorsal and medial raphe project serotonergic afferents to the dorsolateral prefrontal cortex in the monkey, although the dorsal raphe does so more densely and the afferents come particularly from the rostral region. Hence, it was suggested that the innervation of the dorsal raphe could be most closely related with the coordination of the excitability of functionally related cortical areas, whilst the innervation of the medial raphe probably exerts a global influence over cortical activity (Wilson and Molliver, 1991).

Indeed, 28% of the serotonergic terminals afferent to the prefrontal cortex establish synaptic contacts

with spines (predominantly) and with dendritic shafts, while the remainder are made up of free terminals (Smiley and Goldman-Rakic, 1996).

Serotonergic innervation to the prefrontal cortex occurs in all cortical layers, although it is particularly dense in layers 1 and 4. In layers 1, 3 and 5, only 23% of terminals form synapses, which are excitatory on the dendritic shafts of interneurons, and of these 23%, 8% establish synapses with dendritic shafts on pyramidal neurons (Smiley and Goldman-Rakic, 1996).

In the prefrontal cortex, 1A and 2A receptors are expressed in high density (Pazos and Palacios, 1985; Pazos et al., 1985) the 5-HT receptors being the most abundant receptors in this brain region (Pazos et al., 1985; Pompeiano et al., 1994). In this neocortical region, the 1A receptors are inhibitory whereas the 2A receptors are excitatory (Pazos et al., 1985; Pompeiano et al., 1994).

The 5HT_{1A} receptors are present in 60% of the prefrontal pyramidal neurons and in 25% of the GABAergic interneurons (Santana et al., 2004). It has been shown that the 1A 5-HT receptors are involved in the process of learning acquisition and that antagonist blockade of these receptors reverts some cognitive deficiencies induced pharmacologically in monkeys (Harder and Ridley, 2000) and rats (Misane and Ogren, 2003).

In the pyramidal cells, the 5-HT_{1A} receptors are preferentially located in the soma and in the basal dendrites (Riad et al., 2000), whereas 5HT_{2A} receptors establish synaptic contacts with apical dendrites (Xu and Pandey, 2000). Although these neurons are simultaneously excitatory and inhibitory, the inhibitory response predominates, perhaps due to the localization of the type 1A receptors in the axon hillock (Puig et al., 2004b). Furthermore, 30% of the raphe innervation to the cortex comes from GABAergic terminals (Jankowski and Sesack, 2004), which suggests that not only the 5HT_{1A} receptors, but also the GABA(A) receptors might be responsible for the inhibitory responses induced in pyramidal cells after electrical stimulation of the raphe nucleus (Puig et al., 2004a).

From in vitro and in vivo studies in rats, the predominant effect of 5HT in the prefrontal cortex is known to be inhibitory (Jacobs and Azmitia,

1992), despite the high density 5HT₂ of receptors (Lakoski and Aghajanian, 1985). Accordingly, human neocortical neurons could be hyperpolarized via 5HT_{1A} receptors and depolarized in vitro via 5HT₂ receptors (Newberry et al., 1999).

In the prefrontal cortex, 5HT_{2A} receptors are localized postsynaptically on the spines of pyramidal neurons and on the dendrites of both pyramidal neurons and of GABAergic interneurons (Miner et al., 2003) although their proportional density is greater in pyramidal neurons (Willins et al., 1997). Additionally, few presynaptic contacts have been observed in monoaminergic terminals and rarely in glutamatergic terminals (Miner et al., 2003).

The in vitro activation of 5HT_{2A} receptors located in GABAergic interneurons induces both depolarization as well as hyperpolarization (Zhou and Hablitz, 1999). Moreover, it has been reported that electric stimulation of the raphe produces orthodromic excitation of pyramidal cells in the prefrontal cortex, which indicates that the endogenous 5HT is capable of stimulating these receptors in vivo (Puig et al., 2004a).

More than 90% of the prefrontal 5HT₃ receptors are expressed in GABAergic interneurons (Morales et al., 1996). It has been proposed that 5HT modulates pyramidal activity through 5HT₂ receptors in the apical dendrites of pyramidal neurons, as well as through 5HT₃ receptors located in GABAergic interneurons (Zhou and Hablitz, 1999; Puig et al., 2004a).

Most serotonergic terminals in the hippocampal formation are located in the subiculum and CA1, with respect to the dentate gyrus. In the subiculum and CA1, serotonergic innervation is more abundant in the striatum radiatum than in the stratum oriens. With respect to the pyramidal cell layer, there is particularly dense innervation of the molecular stratum of the subiculum and of the CA1, as well as the stratum oriens of the CA3. However, the granular layer of the dentate gyrus possesses a much less dense innervation than the molecular layer and the layer of polymorphic cells (Oleskevich and Descarries, 1990).

The lesion of serotonergic pathways in the hippocampus produces deficiencies in the reference memory of rats, as evaluated in the Morris water maze (Sprague et al., 2003).

The hippocampus possesses 5-HT receptors of the 1A, 1D, 1E, 2C, 3, 5A, 6 and 7 types (Barnes and Sharp, 1999). In a similar way as in the prefrontal cortex, 5-HT directly inhibits pyramidal neurons in the hippocampus via 5HT_{1A} receptors, and indirectly by facilitating GABA release by interneurons through 5HT₃ receptor activation (Burnet et al., 1995). By contrast, the activation of 5HT_{2A} and 5HT_{2C} receptors induces the depolarization of these neurons (Barnes and Sharp, 1999). Due to this effect, 5HT is thought to influence hippocampus-dependent cognitive tasks through the global hyperpolarization effect mediated by 5HT_{1A} receptors (Meeter et al., 2006).

The restriction of dietary tryptophan produces impairments in spatial learning (Olvera-Cortés et al., 1998). This effect could be related to the activity of 5-HT_{1A} receptors, since the impairment of spatial memory has been reported after their activation in the dorsal hippocampus (Egashira et al., 2006). Accordingly, it has been reported that the postsynaptic blockade of the 5HT_{1A} receptor favours efficient retention of a learned task, as well as spatial working memory and of non-spatial reference memory by facilitating the acetylcholine release (Millan et al., 2004).

Both the activation of the 5-HT_{1A} receptor (Seibell et al., 2003) and the generalized depletion of 5-HT induced by the intracerebral ventricular infusion of 5,7-dihydroxytryptamine produces a deterioration in the capacity of spontaneous alternation (Hritcu et al., 2007). However, the chronic restriction of dietary tryptophan improves efficiency in the T-maze (González-Burgos et al., 1995). This apparent contradiction could be explained by a deterioration of the short-term memory due to the facilitatory effect of the cholinergic activity after the blockade of the 5-HT_{1A} receptor (Millan et al., 2004), and because of the diminished cholinergic activity in the hippocampus due to dietary tryptophan restriction (Del Angel-Meza et al., 2003). Thus, the decrease in the cholinergic activity produced by the unavailability of 5-HT could sustain the greater spontaneous alternation under conditions of tryptophan restriction. To corroborate this hypothesis, the nature of the activity of the 5-HT_{1A} receptor within the hippocampus must be established.

In the neostriatum, 10–13% of the serotonergic axons establish excitatory contacts with a similar proportion of dendrites and spines, as well as with other axons. The remaining 75% are associated with free terminals (Soghomonian et al., 1989).

Diverse regions of the striatum possess different densities of 5-HT receptors. Receptors of the 1B, 1D (present mainly in both serotonergic and non-serotonergic presynaptic terminals), 1E, 1F, 2 and 6 subtype exist (Barnes and Sharp, 1999), although in the case of the 5-HT_{1E} and 5-HT_{1F} receptors in particular, there is no evidence of their possible functional roles (Barnes and Sharp, 1999; Stamford et al., 2000).

The application of 5-HT to the posterior striatum, but not to the anterior region, impairs memory consolidation (Prado-Alcalá et al., 2003a). Additionally, in inhibitory avoidance studies the intra-striatal application of a 5-HT₂ receptor blocker produced retrograde amnesia (Prado-Alcalá et al., 2003b). These findings strongly suggest that striatal 5-HT is involved in the consolidation of information related to instrumental learning.

In the cerebral cortex and the hippocampus, there is moderate 5HT₆ receptor expression, which is higher in the striatum. In the striatum, this receptor is located in GABAergic and cholinergic neurons, as well as in thalamic or cortical terminals (Roberts et al., 2002). The activity of the 5HT₆ receptor in the rat striatum impedes the acquisition of instrumental information but does not affect its activity once established (Mitchell et al., 2007).

The striatum is particularly important in the organization of learning and memory that evokes conditioned responses, such as in procedural memory (Sweatt, 2003). Grooming behaviour is characterized by the presence of unitary syntactic chains of stereotypic motor activity present in defined sequences (Berridge and Whishaw, 1992). Alterations in grooming behaviour have been reported in rats subjected to tryptophan restriction (Del Angel-Meza et al., 1996), which could be associated to a disinhibitory effect of striatal dopaminergic activity (Gerson and Baldessarini, 1980). Similarly, it has been reported that the depletion of striatal 5-HT produces facilitation of

egocentric spatial learning and that this effect could be modulated by the activity of DA (Anguiano-Rodríguez et al., 2007).

DA in learning and memory

DA is the most important catecholaminergic neurotransmitter in the Central Nervous System (Bahena-Trujillo and Arias-Montaña, 2000). Like 5-HT, DA is widely distributed in brain regions closely associated with learning and memory processes, including, among others, the prefrontal cerebral cortex, hippocampus and striatum.

The activity of DA is mediated by two families of receptors all coupled to second messengers (Misale et al., 1998) (Table 2). The D₁ subtype is the most abundant dopaminergic receptor in the brain (Jackson and Westlind-Danielsson, 1994); these receptors are found densely distributed in regions related to cognition such as the neostriatum, nucleus accumbens, amygdala, subthalamic nucleus, substantia nigra and the cerebellum. They are also found, but at a moderate density, in the frontal cerebral cortex, thalamus and the globus pallidus, although they are scarce in the hippocampal formation and septal region (Jackson and Westlind-Danielsson, 1994). The other member of the D₁ family, D₅, is less abundant than the D₁ subtype and is restricted to the hippocampus, mammillary nucleus of the hypothalamus and the parafascicular nucleus of the thalamus (Jaber et al., 1996). A high density of the D₂ receptor subtype has been detected in GABAergic striato-pallidal neurons of the neostriatum, in the molecular layer of the hippocampal formation and in the nucleus accumbens. It has also been detected in moderate quantities in the substantia nigra, prefrontal cerebral cortex, globus pallidus,

Table 2. Dopamine receptors

Family	Subtype
D1	D1
	D5
D2	D2
	D3
	D4

amygdala and the thalamus. The D₃ receptor subtype is distributed densely in the septal region, the thalamus and the cerebellum, and a moderate density exists in the parietal cortex, the hippocampal formation, the neostriatum, the nucleus accumbens and the amygdala. The density of these receptors is low in the substantia nigra, frontal cortex, cingulate cortex and the globus pallidus. Finally, the D₄ subtype is highly expressed in the frontal cortex and the amygdala, while it exists in moderate concentrations in the neostriatum and at a low concentration in the hippocampus (Jackson and Westlind-Danielsson, 1994).

There is a high density of D₁ and D₂ receptors in the mouse, rat, guinea pig, cat and monkey basal ganglia. Likewise, the density of D₁ receptors is higher than that of D₂ receptors in the basal ganglia of these species (Camps et al., 1990), and it has been seen that dopaminergic terminals make symmetric contacts (presumably inhibitory) in 67% with dendritic shafts, 30% with dendritic spines and 2–3% with neuronal soma (Descarries et al., 1996).

The concentration of DA receptors in the cerebral cortex displays a high to low gradient from the prefrontal to the occipital cortex (Lidow et al., 1991). The D₁ and D₂ receptors are found in all regions and layers of the rat, cat and monkey cerebral cortex, although the D₂ receptor is more densely expressed in the superficial layers I and II than in the more deep layers in the rat, while their distribution is more homogeneous in the cat and monkey (Richfield et al., 1989). Regarding the dopaminergic innervation afferent to the prefrontal cortex, only 39% form synaptic contacts (Smiley and Goldman-Rakic, 1993). The synaptic dopaminergic contacts in the prefrontal cortex are symmetrical and they occur predominantly on spines of distal dendrites pertaining to pyramidal neurons, as well as on dendritic shafts of GABAergic interneurons, albeit to a lesser extent (Smiley and Goldman-Rakic, 1993). Such symmetric contacts on spines converge with asymmetric excitatory terminals, presumably of glutamatergic nature (Goldman-Rakic et al., 1992; Carr and Sesack, 1996).

Dopaminergic transmission is crucial in the organization of the executive functions mediated

by the working memory in the prefrontal cortex (Luciana and Collins, 1997; García et al., 2005).

Pioneering studies revealed that the depletion of DA produces an impairment of working memory (Brozoski et al., 1979), similar to that produced by electrical over-stimulation (Yang and Seamans, 1996) or over-stimulation of the D₁ receptor in the prefrontal cortex (Zahrt et al., 1997). Thus, the prefrontal activity-mediated working memory is strongly modulated preponderantly by D₁ receptors (Williams and Goldman-Rakic, 1995). Hence, it was proposed that the activation of the existing D₁ in pyramidal neurons provokes a decrease in the temporal dispersion of synaptic entry to these neurons, favouring the appropriate sequences in the activity of the neural networks underlying the behavioural expression of working memory (Surmeier, 2007). Another modulatory mechanism proposed for the dopaminergic activity in the prefrontal cortex involves its effect on the inhibitory activity mediated by GABAergic interneurons. Also, it has been reported that DA can enhance the inhibition of pyramidal neurons mediated by interneurons, through the activation of D₁ receptors located in presynaptic contacts (Kröner et al., 2007).

The functional role of the D₂ receptors in the prefrontal cortex has been less intensely studied. It has been proposed that the phasic release of DA mediates behavioural flexibility through D₂ receptors. The D₂ and not the D₁ receptors, selectively mediate reversal learning without affecting the capacity to learn new stimulus-response associations (Lee et al., 2007).

There are hippocampal afferents to the prefrontal cortex that can form either asymmetric axospine synapses or a lower proportion of asymmetric axodendritic synapses, modulated by dopaminergic synaptic contacts (Carr and Sesack, 1996). In fact, such hippocampal afferents presumably send the contextual spatial information to the prefrontal cortex that underlies the performance in spatial working memory tasks; in addition, those afferents are modulated by the activity of DA receptors (Seamans et al., 1998). It has been reported that the intra-hippocampal application of D₂ receptor agonists improves the performance of spatial working memory, while the

antagonist blockade of these receptors impedes its efficient performance (Wilkerson and Levin, 1999).

The intra-cortical application of D₁ receptor antagonists produces an impaired performance of spatial working memory, both in monkeys (Sawaguchi and Goldman-Rakic, 1991) and rats (Seamans et al., 1995), whilst no effects were observed after the pharmacological manipulation of the D₂ receptor. In particular, retrospective memory is processed in the hippocampus and such information is sent to the prefrontal cortex, allowing it to change a response based on new content to modify the behaviour prospectively. The activity of the prefrontal D₁ receptors modulates the incorporation of retrospective information, while the activation of D₂ receptors participates in the structuring of patterns of future actions (Goto and Grace, 2008).

Through the activation of the D₁ receptors located in pyramidal neurons, the hippocampal dopaminergic system mediates the acquisition of novel information, which can be transformed into long-term memory if it is biologically significant (Lisman and Grace, 2005). Further, it has been demonstrated that the activation of the D₁ receptors are activated during the formation of a persistent memory trace in the hippocampus (O'Carroll et al., 2006), which would be in accordance with the facilitation of the induction of long-term potentiation (LTP) mediated by the stimulation of D₁ receptors (Lemon and Manahan-Vaughan, 2006), and in particular of the D₁ subtype and not the D₅ subtype (Granado et al., 2008).

The role of DA in the striatum is related with the flexibility of the changes in response patterns characteristic of implicit learning and memory processes (O'Neill and Brown, 2007) and of those in which compensation plays an important role. DA release increases in the prefrontal cortex, nucleus accumbens and dorsal striatum when reward is contingent with the learning of a rule that guides a task and during behavioural switching, or when some uncertainty exists (Stefani and Moghaddam, 2006). Likewise, it has been reported that some neural circuits between the prefrontal cortex, the hippocampal formation and diverse regions of the striatum — those underlying

searching behaviour based on memory (Phillips, 2003) — are modulated by an increase of DA release in such regions, and by the chemical decoding of this by D₁ receptors (García et al., 2005).

The nucleus accumbens is part of the ventral striatum. In the dendritic arborization of the medium spiny projection neurons of the accumbens shell region, the dopaminergic fibres coming from the ventral tegmental area are activated non-specifically when a novel environmental stimulus appears. In turn, the DA released modulates the entry of biologically significant information from the prefrontal cortex (attention), the hippocampus (spatial context) and the amygdala (motivation), through D₁ receptors whose function is to maintain a low level of neuronal activity. When the neuron depolarizes after the sustained excitation mediated by cortical, hippocampal and amygdala afferents, the excitatory D₂ receptor contributes to sustaining that depolarizing effect, stabilizing the high state of activity and facilitating the generation of the action potential underlying the transmission of information towards related motor areas, among which the cerebral cortex, globus pallidus, thalamus and the 'core' of nucleus accumbens itself can be found (Fernández-Espejo, 2000). Thus, the acquisition or the extinction of aversive or appetitive conditioning will be modulated by dopaminergic activity differentially mediated by the D₁ and D₂ receptors in the nucleus accumbens.

Dendritic spines in learning and memory

The spines are cytoplasmic elongations disposed perpendicularly to the longitudinal axis of the cell membrane of neurons' dendrites. They measure between 0.1 and 2 µm in length (Harris and Stevens, 1989) and two main anatomical segments are defined in them: the *neck* and the *head* (Harris et al., 1989).

The spines mediate excitatory synaptic transmission postsynaptically and they generally congregate in the dendritic zones farthest away from the soma, whilst the inhibitory synapses are predominantly located in the dendrites close to the soma and generally on dendritic shafts (Edwards, 1995).

Although the spines are not static structures but rather are highly dynamic, they have been classified based on the geometric characteristics of their neck and head. As such, they are initially classified as thin, stubby, mushroom and branched (Harris et al., 1989). This classification has persisted and more recently the categories of double and wide have been added (Tarello-Acuña et al., 2000) (Fig. 2).

The proportional density of each category of spine varies in accordance with the type of neuron in question. However, as a general rule the most abundant spines on a neuron are the thin spines with a density of around 35 to 40%. They are followed by those with a mushroom shape, whose proportional density oscillates between 25 and 35%, while stubby spines represent between 20 and 30%. The proportion of these three types of spines differs from the rest, with wide spines representing 2–5% of the total proportion of spines, while the branched and double types constitute less than 1% of the total (Tarello-Acuña et al., 2000; Lee et al., 2005). The accumulated evidence shows that the proportional density of wide, double and branched spines does not vary as much as that of the thin, mushroom and stubby spines under certain atypical conditions (Pérez-Vega et al., 2000; Tarello-Acuña et al., 2000). This phenomenon might be critical for the integration of the synaptic information they mediate (Pérez-Vega et al., 2000; Kasai et al., 2003).

The head of spines constitutes the site of contact of glutamate-mediated excitatory synapses, and both ionotropic and metabotropic receptors for this neurotransmitter are located in this domain. In a typical excitatory synapse, the ionotropic receptors are more abundant than the metabotropic receptors and they are strongly involved

with the rapid transmission of impulses, while the metabotropic receptors regulate the activity of the others and also mediate the slow effects of glutamate (Dingledine and McBain, 1994). These functions are aided by their localization in the periphery of the postsynaptic density in the head of spines (Luján et al., 1996). In general, both the ionotropic and metabotropic receptors have been related with synaptic plasticity, both transitory and more long lasting (Bennett, 2000; Bortolotto et al., 2005).

Following the release of glutamate by the axon terminal, the activity of the receptors in the head of the spine is generally initiated by the opening of the ion channel coupled to the AMPA receptor, which is permeable to the reciprocal and inverse flux of sodium and potassium. The small difference in the potential generated by this ionic current flow gives rise to a microenvironment that favours the removal of the magnesium coupled to the NMDA receptor from the interior of the ion channel, and its consequent opening due to its voltage sensitivity. This channel is permeable to sodium and potassium such as that coupled to AMPA and in addition is permeable to calcium ions (Bear et al., 1998). Under specific conditions, metabotropic receptors are secondarily activated (Luján et al., 1996) inducing the liberation of calcium from the smooth endoplasmic reticulum (spine apparatus) in the interior of the large spines like the mushroom (Spacek and Harris, 1997), as well as the calcium chelating systems (for example calbindin) in smaller spines such as the thin spines (Ellisman et al., 1990). This process is reversible, and the systems involved in the liberation of calcium are the same as those that 'capture' calcium to avoid damage due to an abnormal intracellular increase in the concentrations of this ion (Ellisman et al., 1990).

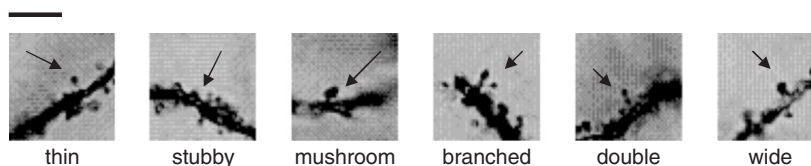


Fig. 2. Photomicrographs of the different types of spines (arrows) impregnated with a modification (González-Burgos et al., 1992) of the Golgi method. Scale bar: 5 μ m. Adapted with permission from González-Burgos et al. (2005).

Whilst the neck is the narrowest part of the spines, the calcium ions find most physical and electrical resistance in their passage from the spine towards the dendrite itself (Volfovsky et al., 1999; Majeuska et al., 2000). Accordingly, in spines with a thin neck such as the thin and mushroom spines, the inversion of the membrane potential increases after the synaptic stimulus (Koch et al., 1992), which favours the entry of calcium through the voltage activated ion channels (Sabatini and Svoboda, 2000). Accordingly, spines with a thin and narrow neck are more efficient in transmitting the synaptic impulses (Koch et al., 1992).

In spines where there is no distinction between the neck and the head, such as the stubby and wide spines, the passage of non-trapped calcium is unrestricted. This would suggest that these classes of spines could be more closely related to the regulation of neuronal excitability (Pérez-Vega et al., 2000; Feria-Velasco et al., 2002), given their effect following over-stimulation (Harris and Kater, 1994; Pérez-Vega et al., 2000).

Spines can display dynamical structural modifications in a matter of minutes (Muller et al., 2000). These interconversions depend on synaptic events that reveal a high degree of specialization that can be expressed under diverse normal or psychopathological conditions. In fact, such dynamic events constitute the morphofunctional substrate that sustains the capacity of synaptic plasticity underlying phenomena as complex as learning and memory (Muller et al., 2000; Pérez-Vega et al., 2000; Kasai et al., 2003; Lee et al., 2005).

The geometric characteristics of each spine type determine the destiny of the synaptic impulse that stimulates them and the type of synaptic stimulus can in turn induce modifications in the geometry of spines (Harris and Kater, 1994). For example, it has been reported that excessive stimulation provokes the retraction and/or disappearance of spines (Jiang et al., 1998) and that by contrast, a weak stimulation produces an increase in the total density of spines (Kirov and Harris, 1999).

There is also a close relationship between the geometric shape of spines and the differential processing of the afferent information (Koch and Zador, 1992). From the pioneering studies of Bliss

and Lømo (1973), it was proposed that the LTP constitutes a neurophysiological event that sustains the acquisition and consolidation of some types of memory (Bliss and Lømo, 1973; Morris et al., 1986). Recent evidence shows that the induction of LTP on dendritic spines induces diverse effects that will be directly related with the learning of specific tasks (Fedulov et al., 2007). The LTP produces a persistent growth of small spines; large spines are also enlarged, but this is transitory (Matsuzaki et al., 2004). The initial activation of NMDA receptors promotes action-mediated changes in the cytoskeleton of the postsynaptic density, the same as those stabilized by the insertion of AMPA receptors recently synthesized in the nucleus, as a response to the initial stimulation; their activity is translated into sustained LTP, making memory consolidation possible (Lamprecht and LeDoux, 2004). This is congruent with the proposal that thin spines are mostly related to the acquisition of information (learning), whilst mushroom spines are related to the storage of such information (memory) (Kasai et al., 2003; Bourne and Harris, 2007). Likewise, it has been observed that the induction of LTP could lead to the formation of new synapses (Agnihotri et al., 1998) or new spines (Engert and Bonhoeffer, 1999). The formation of perforated synaptic densities (Muller et al., 2000) or branched spines (Moser et al., 1994) has also been reported to be a result of LTP, and it has been proposed that these two phenomena could be related. The induction of LTP on thin spines will produce the perforation of the synapse and subsequently, the division of the spine into two new ones through the intermediate and transitory formation of a branched spine or of a denser spine (Muller et al., 2000). Hence, branched spines will constitute a relatively ephemeral transition stage of thin spines to mushroom spines, which could at least in part explain their low density.

The functional repercussions of the regulation of the entry of excitatory information by dendritic spines has been shown in earlier studies that demonstrate a close relationship between synaptic stimulation, the cytoarchitecture of dendritic spines and the behavioural expression of diverse tasks that imply information processing (Pérez-Vega et al.,

2000). Moreover, there is evidence that the density of spines in the dendrites of pyramidal neurons in the CA1 field of the hippocampus of rats increases causally as a result of the formation and expression of the associative memory (Leuner et al., 2003).

There is experimental evidence that the depletion of prefrontal 5-HT produces significantly more efficient behavioural performance in short-term memory tasks, concomitant to a proportional increment of thin and mushroom spines (Pérez-Vega et al., 2000). Likewise, an increase in the proportion of thin spines in neurons has been reported in the hippocampus during the period of oestrus in female rats (González-Burgos et al., 2005) that is related to a greater capacity of spatial task learning (Warren and Juraska, 1997). Alternatively, an increase in mushroom spines was observed in the hippocampus of rats submitted to global acute ischaemia (González-Burgos et al., 2007), concomitant with the retention of the spatial information acquired (Letechipía-Vallejo et al., 2007). These findings are in accordance with the notion that the geometric structure of spines has a direct relationship with the differential processing of mnemonic information (Kasai et al., 2003; Bourne and Harris, 2007). It has also been proposed that small spines — like the thin ones — could be related with short-term memory, whilst large spines — like the mushroom ones — would be related with long-term memory (Matsuzaki et al., 2004).

5-HT–DA interaction in learning and memory

Our knowledge of the interactive participation of the serotonergic and dopaminergic systems in the control and regulation of cognitive processes or their components is scanty.

There is evidence that in the recovery of information related to conditioned responses in a passive avoidance paradigm in rats, the activity of DA is involved in mechanisms of information processing that determine the behavioural strategy, while 5-HT activity is more closely related with the emotional mechanisms that underlie memory (Molodtsova, 2006). In this type of learning, reinforcement is fundamentally important. Thus, changes in the concentrations of DA and 5-HT

have been observed in the hippocampus, prefrontal cortex, amygdala and some thalamic nuclei (considered as ‘cognitive’ areas), as well as in the nucleus accumbens, ventral tegmental area and the amygdala (considered as ‘reward’ areas), following feeding as related to behavioural reinforcement. There was an increase in extracellular DA in the nucleus accumbens, ventral tegmental area, amygdala and the thalamus, whilst it diminished in the hippocampus and the prefrontal cortex. 5-HT diminished in all the areas studied. Thus, brain regions associated with cognition and behavioural reinforcement are activated concomitantly with activation of both dopaminergic and serotonergic activity. Based on this, during the processing of information related to food reward, brain areas related with cognition are also involved. The available evidence suggests that cognitive activity is strongly involved in the brain activity associated to reward in paradigms of conditioned responses, and that both 5-HT as well as DA participate together through mechanisms that have still to be studied in depth (Fallon et al., 2007).

The density of the D₁ and 5-HT₁ receptors is high in prefrontal layers I, II and III, while a large number of 5-HT₂ receptors exist in layers III and IV, and D₂ receptors are located in layer V (Goldman-Rakic et al., 1990). Given that spines are mostly concentrated in the most distal dendritic portions from the soma (Globus and Scheibel, 1966; Valverde, 1967), it is relevant that such spines are the target of dopaminergic and serotonergic terminals (Goldman-Rakic et al., 1989) which integrate the cognitive functions mediated by these two neurotransmitters.

There are reports that prefrontal dopaminergic activity is related to the control of D₁ receptors-mediated attention and working memory functions. Similarly, 5-HT exerts effects over reversal learning in monkeys and humans, and over impulsivity in rats (Robbins, 2005). It has been demonstrated that DA differentially affects the ‘working’ component of short-term memory, and that in the striatum both DA and 5-HT are released only in relation to the working memory component (Karakuyu et al., 2007).

There is evidence that DA release is mediated by serotonergic activity both in the prefrontal cortex

(where it is stronger) as well as in the striatum, and that this is modulated by the activation of the 5-HT_{1B} receptor (Iyer and Bradberry, 1996). Indeed, intracerebral application of 5-HT induces a rise of extracellular DA levels (Iyer and Bradberry, 1996). Similarly, it has been suggested that the 5-HT_{1A} receptors will act on prefrontal glutamatergic pyramidal neurons that project towards the ventral tegmental area, thereby regulating DA release in the cortex (Di Pietro and Seamans, 2007). Additionally, it has been proposed that the 5-HT_{2A} receptors could also be involved in this process (Beique et al., 2007).

In the prefrontal cortex, stimulation of the 5-HT_{2A} receptors could be related with the tonic facilitation of the activity of pyramidal neurons related to the neuronal responses during all stages of the tests of working memory. By contrast, the stimulation of the D₁ DA receptors, selectively suppresses the processing of mnemonic information that underlies working memory, which takes place in the spines of distal dendrites of pyramidal neurons where the majority of the receptors D₁ are located (Williams et al., 2002).

The activation of the 5-HT_{1A} receptors increases DA release in the prefrontal cortex and hippocampus, but not in the striatum or the nucleus accumbens (Sakaue et al., 2000). Thus, it has been reported that the endogenous serotonergic activity in the striatum produces release of DA in awake animals (Yadid et al., 1994) and that the depletion of striatal 5-HT facilitates egocentric learning, which is dependent on dopaminergic modulation (Anguiano-Rodríguez et al., 2007). Accordingly, it has been reported that the endogenous 5-HT in the striatum does not influence DA release in basal conditions, but striatal DA activity is modulated positively by 5-HT when nigrostriatal dopaminergic transmission is activated (Lucas et al., 2000).

It has been reported that 5-HT₃ receptors selectively control DA release dependent on synaptic activity in the striatum, only when the dopaminergic and serotonergic activity increases concomitantly (Porrás et al., 2003). In addition, there is evidence that 5-HT exerts a facilitatory influence on DA release in the striatum, through the activation of the 5-HT₄ receptors (Bonhomme et al., 1995) both in vivo and in vitro (Steward et al., 1996). Indeed, the

presynaptic DA reuptake sites could also be involved in this process (De Deurwaerdère et al., 1996), as well as, in a secondary manner, the 5-HT₁ and 5-HT₂ receptors (Santiago et al., 1998).

Both the blockade of the D₂ receptors as well as the depletion of striatal DA prevents the increase in 5-HT release that accompanies the behavioural activation characteristic of cognitive performance (Mendlin et al., 1999). In particular, the activity of the 5-HT_{2A} receptors participates in the facilitatory control that 5-HT exerts on DA release in the nucleus accumbens. By contrast, the 5-HT_{2B/2C} receptors tonically inhibit basal DA release both in the striatum as well as in the nucleus accumbens itself (De Deurwaerdère and Spampinato, 1999).

Concluding remarks

The activity of 5-HT and DA has been widely studied in relation to the organization, control and behavioural expression of learning and memory. In particular, the modulatory activity of these neurotransmitters by a diverse group of receptors is well defined, albeit its mechanisms of action are still poorly understood.

There are relatively few studies relating the 5-HT–DA interaction to learning and memory, although the interpretation of the data available indicates a mutual modulation between both neurotransmitter systems. The physiological characteristics and functional significance of such interactions depend on the brain region involved, the cognitive component evaluated, and the behavioural paradigm used.

Morphologically, learning and memory processes are sustained, at least in part, by the plastic changes that occur in the dendritic spines of the neurons implicated. There is sufficient information to postulate that the expression of such changes is influenced by the activity of excitatory and inhibitory neurotransmitter systems that, in turn are modulated by the activity of the dopaminergic and serotonergic systems.

The physiological activity of 5-HT and DA is still far from being fully understood, as are the interactions between both these neurotransmitter systems, making it difficult to characterize the

relationships they establish with the organization, control and expression of the diverse components involved in learning and memory processes. However, the conducting of molecular, physiological, morphological and behavioural correlative studies would be very useful for a better understand of the neurobiological basis underlying the functional organization of these cognitive processes.

Abbreviations

5-HT	5-hydroxytryptamine
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
CA1	hippocampal cornus ammonis 1 subfield
CA3	hippocampal cornus ammonis 3 subfield
DA	dopamine
GABA	gamma-aminobutyric acid receptor
LTP	long-term potentiation
NMDA	N-methyl D-aspartate receptor

References

- Agnihotri, N., López-García, J.C., Hawkins, R.D. and Arancio, O. (1998) Morphological changes associated with long-term potentiation. *Histol. Histopathol.*, 13: 1155–1162.
- Anguiano-Rodríguez, P.B., Gaytán-Tocavén, L. and Olvera-Cortés, M.E. (2007) Striatal serotonin depletion facilitates rat egocentric learning via dopamine modulation. *Eur. J. Pharmacol.*, 556: 91–98.
- Baddeley, A. (2000) Short-term and working memory. In: Tulving E. and Craik F.I.M. (Eds.), *The Oxford Handbook of Memory*. Oxford University Press, Oxford, pp. 77–92.
- Bahena-Trujillo, R. and Arias-Montaña, J.A. (2000) Dopamina: síntesis, liberación y receptores en el Sistema Nervioso Central. *Rev. Biomed.*, 11: 39–60.
- Barnes, N.M. and Sharp, T. (1999) A review of central serotonin receptors and their function. *Neuropsychopharmacology*, 38: 1083–1152.
- Başar, E. (2004) Memory and Brain Dynamics. Oscillations Integrating Attention, Perception, Learning, and Memory. CRC Press, USA, pp. 221–229.
- Bear, M.F., Connors, B.W., Paradiso, M.A. (1998) *Neurociencia. Explorando el cerebro*. MASSON-Williams & Wilkins, Barcelona, pp. 68–90.
- Beique, J.C., Imad, M., Mladenovic, L., Gingrich, J.A. and Andrade, R. (2007) Mechanisms of the 5-hydroxytryptamine 2A receptor-mediated facilitation of synaptic activity in prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 104: 9870–9875.
- Bell, C., Abrams, J. and Nutt, D. (2001) Tryptophan depletion and its implications for psychiatry. *Br. J. Psychiatry*, 178: 399–405.
- Bennett, M.R. (2000) The concept of long-term potentiation of transmission at synapses. *Prog. Neurobiol.*, 60: 109–137.
- Berridge, K.C. and Whishaw, I.Q. (1992) Cortex, striatum and cerebellum: control of serial order in a grooming sequence. *Exp. Brain Res.*, 90: 275–290.
- Bliss, T.V. and Lømo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol.*, 232: 331–356.
- Bonhomme, N., De Deurwaerdère, P., Le Moal, M. and Spampinato, U. (1995) Evidence for 5-HT₄ receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology*, 34: 269–279.
- Bortolotto, Z.A., Collett, V.J., Conquet, F., Jia, Z., van der Putten, H. and Collingridge, G.L. (2005) The regulation of hippocampal LTP by the molecular switch, a form of metaplasticity, requires mGlu5 receptors. *Neuropharmacology*, 49: 13–25.
- Bourne, J. and Harris, K.M. (2007) Do thin spines learn to be mushroom spines that remember? *Curr. Opin. Neurobiol.*, 17: 1–6.
- Brozoski, T., Brown, R.M., Rosvold, H.E. and Goldman, P.S. (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science*, 205: 929–931.
- Burnet, P.W.J., Eastwood, S.L., Lacey, K. and Harrison, P.J. (1995) The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res.*, 676: 157–168.
- Camps, M., Kelly, P.H. and Palacios, J.M. (1990) Autoradiographic localization of dopamine D₁ and D₂ receptors in the brain of several mammalian species. *J. Neural Transm. Gen. Sect.*, 80: 105–127.
- Carr, D.B. and Sesack, S.R. (1996) Hippocampal afferents to the rat prefrontal cortex: synaptic targets and relation to dopamine terminals. *J. Comp. Neurol.*, 369: 1–15.
- Cassel, J.C. and Jeltsch, H. (1995) Serotonergic modulation of cholinergic function in the central nervous system: cognitive implications. *Neuroscience*, 69: 1–41.
- Clarke, H.F., Dalley, J.W., Crofts, H.S., Robbins, T.W. and Roberts, A.C. (2004) Cognitive inflexibility after prefrontal serotonin depletion. *Science*, 304: 878–880.
- Costall, B. and Naylor, R.J. (1997) *Neuropharmacology of 5-HT₃ receptors ligands*. In: Baumgarten H.G. and Gøther M. (Eds.), *Serotonergic Neurons and 5-HT Receptors in the CNS*. Springer, Berlin, pp. 409–438.
- Dawson, L.A., Nguyen, H.Q. and Li, P. (2001) The 5-HT₆ receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. *Neuropsychopharmacology*, 25: 662–668.
- De Deurwaerdère, P., Bonhomme, N., Lucas, G., Le Moal, M. and Spampinato, U. (1996) Serotonin enhances striatal

- dopamine outflow in vivo through dopamine uptake sites. *J. Neurochem.*, 66: 210–215.
- De Deurwaerdère, P. and Spampinato, U. (1999) Role of serotonin(2A) and serotonin(2B/2C) receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.*, 73: 1033–1042.
- Del Angel-Meza, A.R., Adame-González, I.G., Segura, J., Montes, R., González-Burgos, I. and Beas-Zárate, C. (2003) Cerebral cholinergic neurotransmission in protein and tryptophan-restricted adult rats. In: Allegri G., Costa C.V.L., Raggazi E., Steinhart H. and Varesio L. (Eds.), *Advances in Experimental Medicine and Biology*, vol. 527. Kluwer Academic Plenum Publishers, New York, pp. 415–421.
- Del Angel-Meza, A.R., González-Burgos, I., Olvera-Cortés, E. and Feria-Velasco, A. (1996) Chronic tryptophan restriction disrupts grooming chain completion in the rat. *Physiol. Behav.*, 59: 1099–1102.
- Descarries, L., Watkins, K.C., Garcia, S., Bosler, O. and Doucet, G. (1996) Dual character, asynaptic and synaptic, of the dopamine innervation in adult rat neostriatum: a quantitative autoradiographic and immunocytochemical analysis. *J. Comp. Neurol.*, 375: 167–186.
- Di Pietro, N.C. and Seamans, J.K. (2007) Dopamine and serotonin interactions in the prefrontal cortex: insights on antipsychotic drugs and their mechanisms of action. *Pharmacopsychiatry*, 40: s27–s33.
- Dingledine, R. and McBain, Ch.J. (1994) Excitatory amino acid transmitters. In: Siegel G.J., Agranoff B.W., Albers R.W. and Molinoff P.B. (Eds.), *Basic Neurochemistry. Molecular, Cellular, and Medical Aspects* (Fifth edition). Raven Press, New York, pp. 367–387.
- Edwards, F.A. (1995) Anatomy and electrophysiology of fast synapses lead to a structural model for long-term potentiation. *Physiol. Rev.*, 75: 759–787.
- Egashira, N., Yano, A., Ishigami, N., Mishima, K., Iwasaki, K., Fujioka, M., Matsushita, M., Nishimura, R. and Fujiwara, M. (2006) Investigation of mechanisms mediating 8-OH-DPAT-induced impairment of spatial memory: involvement of 5-HT_{1A} receptors in the dorsal hippocampus in rats. *Brain Res.*, 1069: 54–62.
- Eglen, R.M., Wong, E.H., Dumis, A. and Bockaert, J. (1995) Central 5-HT₄ receptors. *TIPS*, 16: 391–398.
- Ellisman, M.H., Deering, T.J., Ouyang, Y., Beck, C.F., Tanksley, S.J., Walton, P.D., Airey, J.A. and Sutko, J.L. (1990) Identification and localization of ryanodine binding proteins in the avian central nervous system. *Neuron*, 5: 135–146.
- Engert, F. and Bonhoeffer, T. (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature*, 399: 66–70.
- Fallon, S., Shearman, E., Serhsen, H. and Lajtha, A. (2007) Food reward-induced neurotransmitter changes in cognitive brain regions. *Neurochem. Res.*, 32: 1772–1782.
- Fedulov, V., Rex, C.S., Simmons, D.A., Palmer, L., Gall, C.M. and Lynch, G. (2007) Evidence that long-term potentiation occurs within individual hippocampal synapses during learning. *J. Neurosci.*, 27: 8031–8039.
- Feria-Velasco, A., Del Angel-Meza, A.R. and González-Burgos, I. (2002) Modification of dendritic development. In: Azmitia, E.C., DeFelipe, J., Jones, E.G., Rakic, P., Ribak, C.E. (Eds.), *Changing Views of Cajal's neuron. Progress in Brain Research Series*, Elsevier, USA, Vol. 136, pp. 135–143.
- Fernández-Espejo, E. (2000) ¿Cómo funciona el *nucleus accumbens*? *Rev. Neurol.*, 30: 845–849.
- Fuster, J.M. (1997) Network memory. *TINS*, 20: 451–459.
- García, F.B., Pedraza, C. and Navarro, J.F. (2005) Implicación de la dopamina en los procesos cognitivos del aprendizaje y la memoria. *Psiqu. Biol.*, 12: 232–236.
- Gerard, C., Mestikawi, S., Lebrand, C., Adrien, J., Ruat, M., Traiffort, E., Hamon, M. and Martres, M.P. (1996) Quantitative RT-PCR distribution of serotonin 5-HT₆ receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse*, 23: 164–173.
- Gerson, S.C. and Baldessarini, R.J. (1980) Motor effects of serotonin in the central nervous system. *Life Sci.*, 27: 1435–1451.
- Globus, A. and Scheibel, A.B. (1966) Loss of dendritic spines as an index of presynaptic terminal patterns. *Nature*, 212: 463–465.
- Goldman-Rakic, P.S., Leranth, C., Williams, M.S., Mons, N. and Geffard, M. (1989) Dopamine innervation of pyramidal neurons in primate frontal cortex. *Proc. Natl. Acad. Sci. U.S.A.*, 86: 9015–9019.
- Goldman-Rakic, P.S., Lidow, M.S. and Gallagher, D.W. (1990) Overlap of dopaminergic, adrenergic, and serotonergic receptors and complementarity of their subtypes in primate prefrontal cortex. *J. Neurosci.*, 10: 2125–2138.
- Goldman-Rakic, P.S., Lidow, M.S., Smiley, J.F. and Williams, M.S. (1992) The anatomy of dopamine in monkey and human prefrontal cortex. *J. Neural Trans. Suppl.*, 36: 163–177.
- González-Burgos, I., Alejandro-Gómez, M. and Cervantes, M. (2005) Spine-type densities of hippocampal CA1 neurons vary in proestrus and estrus rats. *Neurosci. Lett.*, 379: 52–54.
- González-Burgos, I., Letechipia-Vallejo, G., López-Loeza, E., Morali, G. and Cervantes, M. (2007) Long-term study of dendritic spines from hippocampal CA1 pyramidal cells, after neuroprotective melatonin treatment following global cerebral ischemia in rats. *Neurosci. Lett.*, 423: 162–166.
- González-Burgos, I., Olvera-Cortés, E., Del Angel-Meza, A.R. and Feria-Velasco, A. (1995) Serotonin involvement in the spontaneous alternation ability: a behavioral study in tryptophan-restricted rats. *Neurosci. Lett.*, 190: 143–145.
- González-Burgos, I., Pérez-Vega, M.I., del Angel-Meza, A.R. and Feria-Velasco, A. (1998) Effect of tryptophan restriction on short-term memory. *Physiol. Behav.*, 63: 165–169.
- González-Burgos, I., Tapia-Arizmendi, G. and Feria-Velasco, A. (1992) Golgi method without osmium tetroxide for the study of the central nervous system. *Biotech. Histochem.*, 67: 288–296.
- Goto, Y. and Grace, A.A. (2008). Dopamine modulation of hippocampal prefrontal cortical interaction drives memory-guided behaviour. *Cereb. Cortex.*, 18: 1407–1414.
- Granado, N., Ortiz, O., Suárez, L.M., Martín, E.D., Ceña, V., Solís, J.M. and Moratalla, R. (2008) D₁ but not D₅ dopamine

- receptors are critical for LTP, spatial learning, and LTP-induced arc and zif268 expression in the hippocampus. *Cereb. Cortex.*, 18: 1–12.
- Harder, J.A. and Ridley, R.M. (2000) The 5-HT_{1A} antagonist WAY 100 635 alleviates cognitive impairments induced by dizocilpine (MK-801) in monkeys. *Neuropharmacology*, 39: 547–552.
- Harris, K.M., Jensen, F.E. and Tsao, B.H. (1989) Ultrastructure, development, and plasticity of dendritic spine synapses in area CA1 of the rat hippocampus: extending our vision with serial electron microscopy and three-dimensional analyses. In: Chan-Palay V. and Kohler Ch. (Eds.), *The Hippocampus* — New Vistas. Alan R. Liss, USA, pp. 33–52.
- Harris, K.M. and Kater, S.B. (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annu. Rev. Neurosci.*, 17: 341–371.
- Harris, K.M. and Stevens, J.K. (1989) Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *J. Neurosci.*, 9: 2982–2997.
- Hritcu, L., Clincinschi, M. and Nabeshima, T. (2007) Brain serotonin depletion impairs short-term memory, but not long-term memory in rats. *Physiol. Behav.*, 91: 652–657.
- Iyer, R.N. and Bradberry, C.W. (1996) Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. *J. Pharmacol. Exp. Ther.*, 277: 40–47.
- Jaber, M., Robinson, S., Misale, C. and Caron, M.G. (1996) Dopamine receptors and brain function. *Neuropsychopharmacology*, 35: 1503–1519.
- Jackson, D.M. and Westlind-Danielsson, A. (1994) Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol. Ther.*, 64: 291–369.
- Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. *Physiol. Rev.*, 72: 165–229.
- Jankowski, M.P. and Sesack, S.R. (2004) Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and γ -aminobutyric acid neurons. *J. Comp. Neurol.*, 468: 518–529.
- Jiang, M., Lee, C.L., Smith, K.L. and Swann, J.W. (1998) Spine loss and other persistent alterations of hippocampal pyramidal cell dendrites in a model of early-onset-epilepsy. *J. Neurosci.*, 18: 8356–8368.
- Karakuyu, D., Herold, C., Güntürkün, O. and Diekamp, B. (2007) Differential increase of extracellular dopamine and serotonin in the ‘prefrontal cortex’ and striatum of pigeons during working memory. *Eur. J. Neurosci.*, 26: 2293–2302.
- Kasai, H., Matsuzaki, M., Noguchi, J., Yasumatsu, N. and Nakahara, H. (2003) Structure-stability-function relationships of dendritic spines. *TINS*, 26: 360–368.
- Kirov, S.A. and Harris, K.M. (1999) Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. *Nat. Neurosci.*, 2: 878–883.
- Koch, Ch. and Zador, A. (1992) Dendritic spines: convergence of theory and experiment. *Science*, 256: 973–974.
- Koch, Ch., Zador, A. and Brown, T.H. (1992) Dendritic spines: convergence of theory and experiment. *Science*, 256: 973–974.
- Kröner, S., Krimer, L.S., Lewis, D.A. and Barrionuevo, G. (2007) Dopamine increases inhibition in the monkey dorso-lateral prefrontal cortex through cell type-specific modulation of interneurons. *Cereb. Cortex.*, 17: 1020–1032.
- Lakoski, J.M. and Aghajanian, G.K. (1985) Effects of ketanserin on neuronal responses to serotonin in the prefrontal cortex, lateral geniculate and dorsal raphe nucleus. *Neuropharmacology*, 24: 265–273.
- Lamprecht, R. and LeDoux, J. (2004) Structural plasticity and memory. *Nat. Rev. Neurosci.*, 5: 45–54.
- Lee, B., Groman, S., London, E.D. and Jentsch, J.D. (2007) Dopamine D(2)/D(3) receptors play a specific role in the reversal of a learned visual discrimination in monkeys. *Neuropsychopharmacology*, 32: 2125–2134.
- Lee, K.J., Kim, H. and Rhyu, I.J. (2005) The roles of dendritic spine shapes in Purkinje cells. *The Cerebellum*, 4: 97–104.
- Lemon, N. and Manahan-Vaughan, D. (2006) Dopamine D₁/D₅ receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J. Neurosci.*, 26: 7723–7729.
- Letechipia-Vallejo, G., López-Loeza, E., Espinoza-González, V., González-Burgos, I., Olvera-Cortés, M.E., Morali, G. and Cervantes, M. (2007) Long-term morphological and functional evaluation of the neuroprotective effects of post-ischemic treatment with melatonin in rats. *J. Pineal Res.*, 42: 139–146.
- Leuner, B., Falduto, J. and Shors, T.J. (2003) Associative memory formation increases the observation of dendritic spines in the hippocampus. *J. Neurosci.*, 23: 659–665.
- Lidow, M.S., Goldman-Rakic, P.S., Gallager, D.W. and Rakic, P. (1991) Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [³H]raclopride, [³H]spiperone and [³H]SCH23390. *Neuroscience*, 40: 657–671.
- Lisman, J. and Grace, A. (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron*, 46: 703–713.
- Lucas, G., De Deurwaerdere, P., Porras, G. and Spampinato, U. (2000) Endogenous serotonin enhances the release of dopamine in the striatum only when nigro-striatal dopaminergic transmission is activated. *Neuropharmacology*, 39: 1984–1995.
- Luciana, M. and Collins, P.F. (1997) Dopaminergic modulation of working memory for spatial but not object cues in normal humans. *J. Cognit. Neurosci.*, 9: 330–347.
- Luján, R., Nusser, Z., Roberts, D.B., Shigemoto, R. and Somogyi, P. (1996) Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur. J. Neurosci.*, 8: 1488–1500.
- Majeuska, A., Brown, E., Ross, J. and Yuste, R. (2000) Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization. *J. Neurosci.*, 20: 1722–1734.
- Masaki, D., Yokoyama, C., Kinoshita, S., Tsuchida, H., Nakatomi, K., Yoshimoto, K. and Fukui, K. (2006)

- Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology*, 189: 249–258.
- Matsuzaki, M., Honkura, N., Ellis-Davies, G.C.R. and Kasai, H. (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature*, 429: 761–766.
- Meeter, M., Talamini, L., Schmitt, J.A.J. and Riedel, W.J. (2006) Effects of 5-HT on memory and the hippocampus: model and data. *Neuropsychopharmacology*, 31: 712–720.
- Meltzer, C.C., Smith, G., Dekosky, S.T., Pollock, B.G., Mathis, C.A., Moore, R.Y., Kupfer, D.J. and Reynolds, C.F. (1998) Serotonin in aging, late-life depression and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacology*, 18: 407–430.
- Mendlin, A., Martin, F.J. and Jacobs, B.L. (1999) Dopaminergic input is required for increases in serotonin output produced by behavioural activation: an in vivo microdialysis study in rat forebrain. *Neuroscience*, 93: 897–905.
- Meneses, A. (1998) Physiological, pathophysiological and therapeutic roles of 5-HT systems in learning and memory. *Rev. Neurosci.*, 9: 275–289.
- Meneses, A. (1999) 5-HT system and cognition. *Neurosci. Biobehav. Rev.*, 23: 1111–1125.
- Meneses, A. (2007) Do serotonin (1–7) receptors modulate short and long-term memory? *Neurobiol. Learn. Mem.*, 87: 561–572.
- Meneses, A. and Perez-Garcia, G. (2007) 5-HT_{1A} receptors and memory. *Neurosci. Biobehav. Rev.*, 31: 705–727.
- Millan, M.J., Gobert, A., Roux, S., Porsolt, R., Meneses, A., Carli, M., Di Cara, B., Jaffard, R., Rivet, J.M., Lestage, P., Mocaer, E., Peglion, J.L. and Dekeyne, A. (2004) The serotonin_{1A} receptor partial agonist S15535 [4-(Benzo[dioxan-5-yl)1-(indan-2-yl)piperazine] enhances cholinergic transmission and cognitive function in rodents: a combined neurochemical and behavioural analysis. *J. Pharmacol. Exp. Ther.*, 311: 190–203.
- Miner, L.A., Backstrom, J.R., Sanders-Bush, E. and Sesack, S.R. (2003) Ultrastructural localization of serotonin(2A) receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience*, 116: 107–117.
- Misale, C., Russel, N.S., Robinson, S.W., Jaber, M. and Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, 78: 189–225.
- Misane, I. and Ogren, S.O. (2003) Selective 5-HT_{1A} antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. *Neuropsychopharmacology*, 28: 253–264.
- Mitchell, E.S., Sexton, T. and Neumaier, J.F. (2007) Increased expression of 5-HT₆ receptors in the rat dorsomedial striatum impairs instrumental learning. *Neuropsychopharmacology*, 32: 1520–1530.
- Molodtsova, G.F. (2006) Different roles of dopamine and serotonin in conditioned passive avoidance response of rats. *Zh. Vyssh. Nerv. Deiat. Im. I. P. Pavlova.*, 56: 242–246.
- Morales, M., Battenberg, E., Delecea, L. and Bloom, F.E. (1996) The type 3 serotonin receptor is expressed in a subpopulation of GABAergic neurons in the rat neocortex and hippocampus. *Brain Res.*, 731: 199–202.
- Morris, R.G., Anderson, E., Lynch, G.S. and Baurdy, M. (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl D- aspartate receptor antagonist, AP5. *Nature*, 319: 774–776.
- Moser, M.B., Trommald, M. and Andersen, P. (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. U.S.A.*, 91: 12673–12675.
- Muller, D., Toni, N. and Buchs, P.A. (2000) Spine changes associated with long-term potentiation. *Hippocampus*, 10: 596–604.
- Newberry, N.R., Footitt, D.R., Papanastassiou, V. and Reynolds, D.J.M. (1999) Actions of 5-HT on human neocortical neurons in vitro. *Brain Res.*, 833: 93–100.
- O'Carroll, C.M., Martin, S.J., Sandin, J., Frenguelli, B. and Morris, R.G. (2006) Dopaminergic modulation of the persistence of one-trial hippocampus-dependent memory. *Learn. Mem.*, 13: 760–769.
- Oleskevich, S. and Descarries, L. (1990) Quantified distribution of the serotonin innervation in adult rat hippocampus. *Neuroscience*, 34: 19–33.
- Olvera-Cortés, E., Pérez-Vega, M.I., Barajas-López, G., del Angel-Meza, A.R., González-Burgos, I. and Feria-Velasco, A. (1998) Place learning impairment in chronically tryptophan-restricted rats. *Nutr. Neurosci.*, 1: 223–235.
- O'Neill, M. and Brown, V.J. (2007) The effect of striatal dopamine depletion and the adenosine A2A antagonist KW-6002 on reversal learning in rats. *Neurobiol. Learn. Mem.*, 88: 75–81.
- Pazos, A., Cortés, M. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-2 receptors. *Brain Res.*, 346: 231–249.
- Pazos, A. and Palacios, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res.*, 346: 205–230.
- Pérez-Vega, M.I., Feria-Velasco, A. and González-Burgos, I. (2000) Prefrontocortical serotonin depletion results in plastic changes of prefrontocortical pyramidal neurons, underlying a greater efficiency of short-term memory. *Brain Res. Bull.*, 53: 291–300.
- Peters, J.A., Malone, H.M. and Lambert, J.J. (1992) Recent advances in the electrophysiological characterization of 5-HT₃ receptors. *Trends Pharmacol. Sci.*, 13: 391–397.
- Phillips, A.G. (2003) Mesocorticolimbic dopamine: a neurochemical link between motivation and memory. *Int. Cong. Ser.*, 1250: 509–526.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNA: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Mol. Brain Res.*, 23: 163–178.
- Porras, G., De Deurwaerdere, P., Moison, D. and Spampinato, U. (2003) Conditional involvement of striatal serotonin₃ receptors in the control of in vivo dopamine outflow in the rat striatum. *Eur. J. Neurosci.*, 17: 771–781.

- Prado-Alcalá, R.A., Ruiloba, M.I., Rubio, L., Solana-Figueroa, R., Medina, C., Salado-Castillo, R. and Quitarte, G.L. (2003a) Regional infusions of serotonin into the striatum and memory consolidation. *Synapse*, 47: 169–175.
- Prado-Alcalá, R.A., Solana-Figueroa, R., Galindo, L.E., Medina, A.C. and Quitarte, G.L. (2003b) Blockade of striatal 5-HT₂ receptors produces retrograde amnesia in rats. *Life Sci.*, 74: 481–488.
- Puig, M.V., Artigas, F. and Celada, P. (2004a) Modulation of the activity of pyramidal neurons in rat prefrontal cortex by raphe stimulation in vivo: involvement of serotonin and GABA. *Cereb. Cortex.*, 15: 1–14.
- Puig, M.V., Celada, P. and Artigas, F. (2004b) Control serotoninérgico de la corteza prefrontal. *Rev. Neurol.*, 39: 539–547.
- Riad, M., Garcia, S., Watkins, K.C., Jodoin, N., Doucet, E., Langlois, X., el Mestikawi, S., Hamon, M. and Desacriès, L. (2000) Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *J. Comp. Neurol.*, 417: 181–194.
- Richfield, E.K., Young, A.B. and Penney, J.B. (1989) Comparative distributions of dopamine D-1 and D-2 receptors in the cerebral cortex of rats, cats, and monkeys. *J. Comp. Neurol.*, 286: 409–426.
- Riedel, W.J., Sobczak, S. and Schmitt, J.A. (2003) Tryptophan modulation and cognition. *Adv. Exp. Med. Biol.*, 527: 207–213.
- Robbins, T.W. (2005) Chemistry of the mind: neurochemical modulation of prefrontal cortical function. *J. Comp. Neurol.*, 493: 140–146.
- Roberts, J.C., Reavill, C., East, S.Z., Harrison, P.J., Patel, S., Routledge, C. and Leslie, R.A. (2002) The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT(6) receptor antagonist [(125)I]SB-258585. *Brain Res.*, 934: 49–57.
- Sabatini, B.L. and Svoboda, K. (2000) Analysis of calcium channels in single spines using optical fluctuations analysis. *Nature*, 408: 589–593.
- Sakaue, M., Somboonthum, P., Nishihara, B., Koyama, Y., Hashimoto, H., Baba, A. and Matsuda, T. (2000) Postsynaptic 5-hydroxytryptamine_{1A} receptor activation increases in vivo dopamine release in rat prefrontal cortex. *Br. J. Pharmacol.*, 129: 1028–1034.
- Santana, N., Bortolozzi, A., Serratz, J., Mngod, G. and Artigas, F. (2004) Expression of serotonin_{1A} and serotonin_{2A} receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cereb. Cortex.*, 14: 1100–1109.
- Santiago, M., Matarredona, E.R., Machado, A. and Cano, J. (1998) Influence of serotoninergic drugs on in vivo dopamine extracellular output in rat striatum. *J. Neurosci. Res.*, 52: 591–598.
- Sawaguchi, T. and Goldman-Rakic, P.S. (1991) D₁ dopamine receptors in prefrontal cortex: involvement in working memory. *Science*, 251: 947–950.
- Schiapparelli, L., Simón, A.M., Del Río, J. and Frechilla, D. (2006) Opposing effects of AMPA and 5-HT_{1A} receptor blockade on passive avoidance and object recognition performance: correlation with AMPA receptor subunit expression in rat hippocampus. *Neuropharmacology*, 50: 897–907.
- Schmitt, J., Jorissen, B. and Sobczak, S. (2000) Tryptophan impairs memory consolidation but improves focussed attention in healthy young volunteers. *J. Psychopharmacol.*, 14: 21–29.
- Seamans, J.F., Floresco, S.B. and Phillips, A.G. (1995) Selective impairment on a delayed radial arm task following local administration of a selective D₁, but not a D₂, antagonist into the prefrontal cortex. *Soc. Neurosci. Abstr.*, 21: p. 1942.
- Seamans, J.K., Floresco, S.B. and Phillips, A.G. (1998) D₁ receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J. Neurosci.*, 18(4): 1613–1621.
- Seibell, P.J., Demarest, J. and Rhoads, D.E. (2003) 5-HT_{1A} receptor activity disrupts spontaneous alternation behaviour in adult rats. *Pharmacol. Biochem. Behav.*, 74: 559–564.
- Shirahata, T., Tsunoda, M., Santa, T., Kirino, Y. and Watanabe, S. (2006) Depletion of serotonin selectively impairs short-term memory without affecting long-term memory in odor learning in the terrestrial slug *Limax valentianus*. *Learn. Mem.*, 13: 267–270.
- Smiley, J.F. and Goldman-Rakic, P.S. (1993) Heterogenous targets of dopamine synapses in monkey prefrontal cortex demonstrated by serial section electron microscopy: a laminar analysis using the silver-enhanced diaminobenzidine sulphide (SEDS) immunolabeling technique. *Cereb. Cortex.*, 3: 223–238.
- Smiley, J.F. and Goldman-Rakic, P.S. (1996) Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J. Comp. Neurol.*, 367(3): 431–443.
- Soghomonian, J.J., Descarries, L. and Watkins, K.C. (1989) Serotonergic innervation in adult rat neostriatum. II. Ultrastructural features: a radioautographic and immunocytochemical study. *Brain Res.*, 481: 67–86.
- Spacek, J. and Harris, K.M. (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.*, 17: 190–203.
- Sprague, J.E., Preston, A.S., Leifheit, M. and Woodside, B. (2003) Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol. Behav.*, 79: 281–287.
- Squire, L.R. (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. *Psychol. Rev.*, 99: 195–231.
- Stamford, J.A., Davidson, C., McLaughlin, D.P. and Hopwood, S.E. (2000) Control of dorsal raphe 5-HT function by multiple 5-HT(1) autoreceptors: parallel purposes or pointless plurality? *Trends Neurosci.*, 23: 459–465.
- Staubli, U. and Xu, F.B. (1995) Effects of 5-HT₃ receptor antagonism on hippocampal theta rhythm, memory, and LTP induction in the freely moving rat. *J. Neurosci.*, 15: 2445–2452.

- Stefani, M.R. and Moghaddam, B. (2006) Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. *J. Neurosci.*, 26: 8810–8818.
- Steward, L.J., Ge, J., Stowe, R.L., Brown, D.C., Bruton, R.K., Stokes, P.R. and Barnes, N.M. (1996) Ability of 5-HT₄ receptor ligands to modulate rat striatal dopamine release in vitro and in vivo. *Br. J. Pharmacol.*, 117: 55–62.
- Surmeier, D.J. (2007) Dopamine and working memory mechanisms in prefrontal cortex. *J. Physiol.*, 581: p. 885.
- Sweatt, J.D. (2003) *Mechanisms of Memory* Academic Press, USA, pp. 3–28
- Tarello-Acuña, L., Olvera-Cortés, E. and González-Burgos, I. (2000) Prenatal and postnatal exposure to ethanol induces changes in the shape of the dendritic spines from hippocampal CA1 pyramidal neurons of the rat. *Neurosci. Lett.*, 286: 13–16.
- Valverde, F. (1967) Apical dendritic spines of the visual cortex and light deprivation in the mouse. *Exp. Brain Res.*, 3: 337–352.
- Van der Veen, F.M., Evers, E.A., van Deursen, J.A., Deutz, N.E., Baches, W.H. and Schmitt, J.A. (2006) Acute tryptophan depletion reduces activation in the right hippocampus during encoding in an episodic memory task. *Neuroimage*, 31: 1188–1196.
- Volfovsky, N., Parnas, H., Segal, M. and Korkotian, E. (1999) Geometry of dendritic spines affects calcium dynamics in hippocampal neurons: theory and experiments. *J. Neurophysiol.*, 81: 450–462.
- Warren, S.G. and Juraska, J.M. (1997) Spatial and nonspatial learning across the rat estrous cycle. *Behav. Neurosci.*, 111: 259–266.
- Wilkerson, A. and Levin, E.D. (1999) Ventral hippocampal dopamine D₁ and D₂ systems and spatial working memory in rats. *Neuroscience*, 89: 743–749.
- Williams, G.V. and Goldman-Rakic, P.S. (1995) Blockade of dopamine D₁ receptors enhances memory fields of prefrontal neurons in primate cerebral cortex. *Nature*, 376: 572–575.
- Williams, G.V., Rao, S.G. and Goldman-Rakic, P.S. (2002) The physiological role of 5-HT_{2A} receptors in working memory. *J. Neurosci.*, 22: 2843–2854.
- Willins, D.L., Deutch, A.Y. and Roth, B.L. (1997) Serotonin 5-HT_{2A} receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse*, 27: 79–82.
- Wilson, M.A. and Molliver, M.E. (1991) The organization of serotonergic projections to cerebral cortex in primates: retrograde transport studies. *Neuroscience*, 44: 555–570.
- Xu, T. and Pandey, S.C. (2000) Cellular localization of serotonin(2A) (5HT(2A)) receptors in the rat brain. *Brain Res. Bull.*, 51: 499–505.
- Yadid, G., Pacak, K., Kopin, I.J. and Goldstein, D.S. (1994) Endogenous serotonin stimulates striatal dopamine release in conscious rats. *J. Pharmacol. Exp. Ther.*, 270: 1158–1165.
- Yang, C.R. and Seamans, J.K. (1996) Dopamine D₁ receptor actions in layer V–VI rat prefrontal cortex neurons in vitro: modulation of dendritic-somatic signal integration. *J. Neurosci.*, 16: 1922–1935.
- Yasuno, F. (2004) Hippocampal serotonin 1A receptor and memory function. *Seishin Shinkeigaku Zasshi*, 106: 1314–1322.
- Zahrt, J., Taylor, J.R., Mathew, R.G. and Arnsten, A.F.T. (1997) Supranormal stimulation of D₁ dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J. Neurosci.*, 17: 8528–8535.
- Zhou, F.M. and Hablitz, J.J. (1999) Activation of serotonin receptors modulates synaptic transmission in rat cerebral cortex. *J. Neurophysiol.*, 82: 2989–2999.
- Zifa, E. and Fillion, G. (1992) 5-Hydroxytryptamine receptors. *Pharmacol. Rev.*, 44: 401–458.

CHAPTER 29

The roles of dopamine and serotonin, and of their receptors, in regulating sleep and waking

Jaime M. Monti* and Héctor Jantos

Department of Pharmacology and Therapeutics, School of Medicine, Clinics Hospital, Montevideo 11600, Uruguay

Abstract: Based on electrophysiological, neurochemical and neuropharmacological approaches, it is currently accepted that serotonin (5-HT) and dopamine (DA) function to promote waking (W) and to inhibit slow wave sleep (SWS) and/or rapid-eye-movement sleep (REMS). Serotonergic neurons of the dorsal raphe nucleus (DRN) fire at a steady rate during W, decrease their firing during SWS and virtually cease activity during REMS. On the other hand, DA cells in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) do not change their mean firing rate across the sleep–wake cycle. It has been proposed that DA cells in the midbrain show a change in temporal pattern rather than firing rate during the sleep–wake cycle. Available evidence tends to indicate that during W and REMS an increase of burst firing activity of DA neurons occurs together with an enhanced release of DA in the VTA, the nucleus accumbens and several forebrain structures. Recently, DA neurons were characterised in the ventral periaqueductal grey matter (VPAG) that express Fos protein during W. Lesioning of these cells resulted in an increase of SWS and REMS, which led to the proposal that VPAG DA neurons may play a role in the promotion of W. Systemic injection of full agonists at postsynaptic 5-HT_{1A} (8-OH-DPAT, flesinoxan), 5-HT_{1B} (CGS 12066B, CP-94,253), 5-HT_{2A/2C} (DOI, DOM) and 5-HT₃ (*m*-chlorophenylbiguanide) receptors increases W and reduces SWS and REMS. On the other hand, microdialysis perfusion or direct infusion of 8-OH-DPAT or flesinoxan into the DRN, where somatodendritic 5-HT_{1A} receptors are located, significantly increases REMS. Systemic administration of the selective DA D₁ receptor agonist SKF 38393 induces behavioural arousal together with an increase of W and a reduction of sleep. On the other hand, injection of a DA D₂ receptor agonist (apomorphine, bromocriptine, quinpirole) gives rise to biphasic effects, such that low doses reduce W and augment SWS and REMS whereas large doses induce the opposite effects. Not much is known about dopamine–serotonin interaction in the regulation of sleep and W. It has been shown that VTA and SNc DA neurons and DRN 5-HT neurons influence each other. Thus, depending on the receptor subtype involved, 5-HT either facilitates or inhibits the functioning of DA cells. On the other hand, activation of DA D₂-like receptors in the DRN increases the activity of 5-HT neurons. Thus, it can be speculated that local microinjection of DA and 5-HT ligands into the DRN and the VTA/SNc, respectively, would affect the actions of the corresponding neurons on sleep and W.

Keywords: sleep; waking; REM sleep; serotonin receptors; dopamine receptors; dorsal raphe nucleus; ventral tegmental area; substantia nigra compacta

*Corresponding author. Tel.: +59 82 710 58 07;
E-mail: jmonti@mednet.org.uy

Introduction

The processes resulting in the control of sleep and waking (W) are complex and involve the choline ester acetylcholine, peptides, amino acids, purines and monoamines, among them serotonin (5-HT) and dopamine (DA). Greater understanding of the roles of 5-HT and DA in the modulation of these behavioural states is important for further advances in the treatment of illnesses in which sleep disturbances are prominent, such as schizophrenia and Parkinson's disease (PD). In addition, 5-HT and/or DA play very important roles in the mechanisms of action of most drugs used for the treatment of those conditions (Monti, 2004; Monti and Monti, 2004). Although questions remain, electrophysiological, neurochemical and neuropharmacological studies have revealed much detailed information about the roles of 5-HT and DA in sleep and W.

Brain regions and neurotransmitter systems involved in the regulation of waking, non-REM sleep and REM sleep

The brain regions involved in the promotion of the waking state are located in the (1) brainstem (dorsal raphe nucleus (DRN), median raphe nucleus (MRN), locus coeruleus, laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) and medial pontine reticular formation (mPRF)); (2) hypothalamus (tuberomammillary nucleus and lateral hypothalamus); (3) basal forebrain (medial septal area, nucleus basalis of Meynert) and (4) midbrain (ventral tegmental area (VTA), substantia nigra pars compacta (SNc)) (Pace-Schott and Hobson, 2002; Jones, 2003).

The following neurotransmitters function to promote waking (W): (1) acetylcholine (LDT/PPT, basal forebrain); (2) noradrenaline (locus coeruleus); (3) serotonin (DRN, MRN); (4) histamine (tuberomammillary nucleus); (5) glutamate (mPRF, basal forebrain, thalamus); (6) orexin (lateral hypothalamus) and (7) dopamine (VTA, SNc) (Zoltoski et al., 1999; Monti, 2004). Noradrenaline-, serotonin- and histamine-containing neurons send long ascending projections to the

forebrain and cerebral cortex; DA-containing cells project into the basal ganglia and the prefrontal cortex; cholinergic neurons from the midbrain tegmentum project to the thalamus and the basal forebrain, whereas cholinergic basal forebrain neurons have widespread rostral projections to the cerebral cortex and the hippocampus; orexin-containing cells from the lateral hypothalamus project to the entire forebrain and brainstem arousal systems; and glutamatergic neurons comprise the projection neurons of the mPRF and the thalamus (Baghdoyan and Lydic, 2002; Jones, 2003) (Fig. 1).

Neurons of the basal forebrain, preoptic area and anterior hypothalamus constitute the sleep-inducing system. Electrical stimulation of the preoptic area and the horizontal limb of the diagonal band of Broca leads to sleep with electrocortical synchronization in the cat (Stermann and Clemente, 1962). In contrast, lesions involving the preoptic area and the horizontal limb of the diagonal band of Broca disrupt slow wave sleep (SWS) and rapid-eye-movement sleep (REMS) (Lucas and Stermann, 1975). Recording of single-cell activity in the preoptic/anterior hypothalamic area of the cat and the rat has enabled the identification of neurons that increase their discharge rates during SWS (Alam et al., 1995; Szymusiak et al., 2001). A majority of these neurons contain γ -aminobutyric acid (GABA) and galanin, two inhibitory neurotransmitters, and project to the basal forebrain and to brainstem and hypothalamic areas involved in the promotion of W.

Adenosine has been proposed to induce sleep by inhibiting cholinergic neurons of the basal forebrain and the brainstem. In this respect, adenosine and the adenosine transport inhibitor NBTI decrease the discharge rate of basal forebrain neurons during W, whereas the adenosine A_1 receptor antagonist CPDX induces the opposite effect in the rat (Alam et al., 1999; Strecker et al., 2000). In addition, perfusion of adenosine into the LDT of the cat results in a significant decrease of W and the enhancement of sleep (Portas et al., 1997).

Cholinergic neurons of the LDT/PPT act to promote REMS. The predominantly glutamatergic

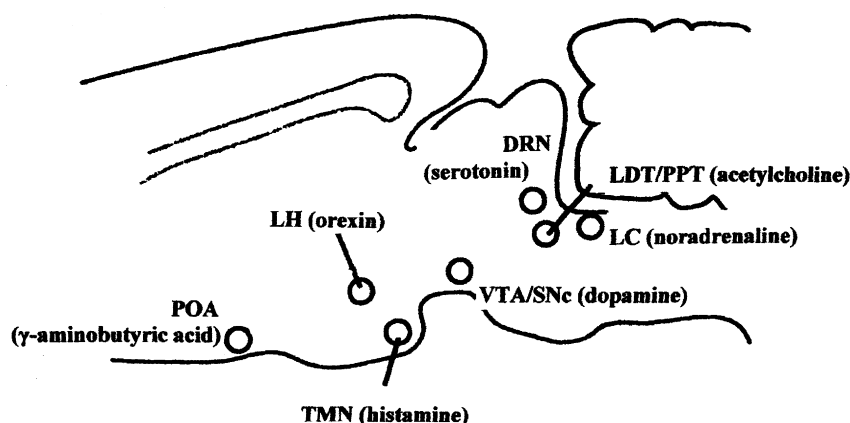


Fig. 1. Neural structures located in the brainstem and the hypothalamus, and neurotransmitters involved in the regulation of sleep and waking. *Abbreviations:* DRN, dorsal raphe nucleus; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LC, locus coeruleus; LH, lateral hypothalamus; POA, preoptic area; VTA/SNc, ventral tegmental area and substantia nigra pars compacta; TMN, tuberomammillary nucleus.

neurons of the REMS-induction region of the mPRF are in turn activated by cholinergic cells, which results in the occurrence of the tonic and the phasic components of REMS (Saper et al., 1997; Baghdoyan and Lydic, 2002). All these neurons are inhibited by serotonergic (DRN), noradrenergic (locus coeruleus), histaminergic (tuberomammillary nucleus), orexinergic (lateral hypothalamus) and dopaminergic (VTA, SNc) cells (McCarley et al., 1995; Mallick et al., 2002).

Thus, as briefly outlined, the control of sleep and W by neurotransmitters is a complex process, and DA and 5-HT only represent one facet of this complicated operation.

Dopaminergic nuclei and pathways

One group of DA neurons arises in the SNc (A_9 cell group) and terminates in the dorsal striatum (caudate-putamen). A second group of DA neurons arises in the VTA (A_{10} cell group) and innervates: (1) limbic areas, including the septal area, olfactory tubercles, nucleus accumbens, amygdaloid complex, hippocampus and piriform cortex (mesolimbic projection) and (2) the medial prefrontal, cingulate and entorhinal areas (mesocortical projection) (Moore and Bloom, 1978). Recently, Lu et al. (2006) characterised DA neurons in the ventral periaqueductal grey matter

(VPAG) of the rat that express Fos protein during natural W. Lesions of these cells with the neurotoxin 6-hydroxydopamine significantly increased SWS and REMS, which led to the proposal that VPAG DA neurons may play a role in the promotion of W.

Afferent and efferent connections of the VTA, the SNc and the VPAG

DA neurons of the VTA, the SNc and the VPAG innervate areas involved in sleep/wake regulation. These areas include the serotonergic cells of the DRN and the MRN, the noradrenergic cells of the locus coeruleus, the cholinergic cells of the LDT/PPT and the basal forebrain, the orexinergic neurons of the lateral hypothalamus, the histaminergic neurons of the posterior hypothalamus and the neurons that modulate the behavioural state in the thalamus and the prefrontal cortex. In turn, inputs to the dopaminergic VTA, SNc and VPAG cells have been found from most of those anatomical structures.

Dopamine receptors

Two distinct groups of DA receptors, D_1 - and D_2 -like receptors, have been characterised. The D_1

subfamily includes the D₁ and D₅ receptors whereas the D₂ subfamily comprises the D₂, D₃ and D₄ receptors.

The D₁ receptor is a postsynaptic receptor. It is coupled to adenylate cyclase, and its stimulation facilitates the activity of the enzyme. Rat brain areas rich in D₁ receptors include the caudate-putamen, the nucleus accumbens and the olfactory tubercle. D₁ receptors are also expressed, at lower levels, in the structures involved in the regulation of the behavioural state, including the cerebral cortex, the thalamus, the limbic system and the hypothalamus (Mansour and Watson, 1995; Meador-Woodruff, 1995). DA-containing neurons of the DRN, the locus coeruleus, the VTA and the SNc do not express D₁ receptor mRNA (Weiner et al., 1991).

The D₅ receptor is also a postsynaptic receptor linked to the activation of adenylate cyclase. Its abundance is much lower than that of the D₁ receptor. The D₅ receptor is mainly localised in the olfactory tubercle, the hippocampus and the hypothalamus. On the other hand, no expression of D₅ receptor mRNA has been reported in the DRN, the locus coeruleus or the LDT/PPT.

DA receptors of the D₂ subfamily are predominantly coupled to the inhibition of adenylate cyclase. In this respect, D₂-like receptors increase outward K⁺ currents, inhibit inward Ca²⁺ currents and modulate the metabolism of phosphoinositide (Missale et al., 1998).

The D₂ receptor is the predominant D₂-like subtype in the brain. The areas of highest expression of the D₂ receptor in the rat brain include the caudate-putamen, the nucleus accumbens and the olfactory tubercle. D₂ receptor mRNA is also present in the cerebral cortex, the basal forebrain, the limbic system (septal region, hippocampus, amygdala), the hypothalamus (tuberomammillary nucleus) and the rhombencephalon (pontine reticular formation, raphe nuclei, locus coeruleus). The D₂ receptor has been characterised also on cell bodies and dendrites in the VTA and the SNc, where it functions as an autoreceptor (Meador-Woodruff, 1995; Missale et al., 1998).

D₃ and D₄ receptors are found at much lower levels and are more narrowly distributed in the

central nervous system compared with the D₂ receptor. The D₃ receptor is mainly expressed in the Islands of Calleja and the nucleus accumbens. D₃ receptor mRNA occurs also at moderate-to-low levels in the cerebral cortex, hippocampus, amygdala, hypothalamus and DRN (Sibley and Monsma, 1992; Meador-Woodruff, 1995). D₃ receptors expressed in the VTA and the SNc presumably are likely to be autoreceptors. No cells expressing D₃ receptor mRNA have been detected in the LDT/PPT or the locus coeruleus (Mansour and Watson, 1995; Emilien et al., 1999).

In the rodent brain, D₄ receptors are expressed in the frontal cortex, amygdala, hippocampus and hypothalamus (Meador-Woodruff, 1995).

Firing pattern of VTA and SNc neurons

DA-containing neurons of the A₉ and A₁₀ groups fire in one of the two patterns: (1) slow, irregular spontaneous action potentials (tonic firing pattern) or (2) bursts of spikes that show a progressive decrease in amplitude and increase in duration (phasic activity) (Grace and Bunney, 1984, 1985). The burst firing is dependant on afferent inputs from several cortical and subcortical structures (Murray et al., 1994).

Grace (2002) recognises a tonic and a phasic component of DA release. The tonic component of DA release takes place when the cells are firing in a slow, irregular, single-spike mode and is spatially diffuse (Gonon, 1988). The phasic component is mediated by burst firing of DA neurons and is characterised by a larger and more efficient release of DA from axons than when these cells fire in an irregular single-spike mode (Gonon, 1988). The bursting activity of DA neurons in the midbrain has been associated with reward mechanisms, locomotor activity and cognitive functions (Le Moal and Simon, 1991; Schultz, 1998). Recently, it was proposed that burst firing could be also related to the occurrence of W and REM sleep (Rye and Jankovic, 2002; Dahan et al., 2007). Opposite to what occurs in serotonergic, noradrenergic and histaminergic neurons, DA cells in the midbrain would show a change in the temporal pattern rather than the firing rate during the sleep-wake

state. The change in temporal pattern would manifest as burst firing and depend on inputs from the prefrontal cortex (excitatory aminoacidergic afferents) (Overton and Clark, 1997); the lateral hypothalamus, where orexinergic neurons are located (Fadel and Deutch, 2002); the LDT/PPT nuclei, where cholinergic neurons have been characterised (Kitai et al., 1999) and the subthalamic nucleus, where glutamatergic neurons have been described (Kitai et al., 1999). Inputs from the DRN and the locus coeruleus have also been proposed to facilitate the occurrence of bursting activity (White, 1996).

Serotonergic nuclei and pathways

Serotonergic neurons of raphe regions of the brainstem form rostral and caudal cell groups. The most rostral cell aggregates innervate the telencephalon, the mesencephalon and the rhombencephalon, whereas the most caudal nuclei project mainly to the medulla and the spinal cord (Cooper et al., 1996; Stanford, 2001). The two major midbrain nuclei contributing ascending serotonergic innervation are the DRN and the MRN. The innervation coming from the DRN and the MRN either overlaps or projects to complementary neuroanatomical structures in the central nervous system (Vertes and Kocsis, 1994; Vertes et al., 1999).

Afferent and efferent connections of the dorsal raphe nucleus and the median raphe nucleus

Serotonergic neurons of the DRN and the MRN innervate brain areas involved in sleep/wake regulation, including the dopaminergic neurons of the SNc/VTA; the cholinergic nuclei of the mesencephalon and the basal forebrain; the noradrenergic cells of the locus coeruleus; the GABAergic, histaminergic and orexinergic cell aggregates of the hypothalamus; and the glutamatergic neurons of the thalamus and the brainstem reticular formation. In turn, inputs to the DRN and the MRN have been found from the basal forebrain, the hypothalamus and the cholinergic,

dopaminergic and noradrenergic nuclei of the brainstem.

Firing pattern of serotonergic neurons

The activity of serotonergic DRN neurons is at its highest during W; it diminishes during SWS and is virtually suppressed when the animal starts REMS (Trulson and Jacobs, 1979).

Serotonin receptors

The 5-HT receptors can be classified into at least seven classes, designated 5-HT₁₋₇. The 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₅ classes consist of five (5-HT_{1A-B-D-E-F}), three (5-HT_{2A-B-C}) and two (5-HT_{3A-B} and 5-HT_{5A-B}) subtypes, respectively, whereas the 5-HT₄, 5-HT₆ and 5-HT₇ classes have at present one subtype each (Hoyer et al., 1994; Baez et al., 1995; Hoyer and Martin, 1996). Except for the 5-HT₃ receptor, all other 5-HT receptors are structurally related to the superfamily of G-protein-coupled receptors.

The 5-HT_{1A} receptor is located on the soma and the dendrites (somatodendritic autoreceptor) of 5-HT neurons and at postsynaptic sites. The 5-HT_{1A} receptor is coupled to adenylate cyclase and its activation inhibits the enzyme. Stimulation of the somatodendritic 5-HT_{1A} receptor inhibits the firing rate of serotonergic neurons, whereas activation of the postsynaptic receptor induces inhibitory responses on target structures (Wang and Aghajanian, 1977; Aghajanian and Lakoski, 1984). Brain areas rich in 5-HT_{1A} receptors include the cerebral cortex, the hippocampus, the septal nuclei, the hypothalamus and some amygdaloid and raphe nuclei, particularly the DRN (Luebke et al., 1992; Austin et al., 1994; Kia et al., 1996a, b; Thakkar et al., 1998).

The 5-HT_{1B} receptor is linked to the inhibition of adenylate cyclase, and is located at presynaptic (5-HT axon terminals) and postsynaptic sites. It is involved in the regulation of synaptic release of 5-HT and of other neurotransmitters, including acetylcholine, noradrenaline, GABA and glutamate, which is indicative of its role as auto- and

heteroreceptor, respectively. Mapping of the 5-HT_{1B} receptor mRNA and its visualization by autoradiography tend to indicate that its distribution is widespread in the central nervous system. In this respect, it has been characterised in the cerebral cortex, limbic system (amygdala, lateral septal nucleus, hippocampus), basal ganglia (accumbens nucleus, caudate-putamen), thalamus, hypothalamus (lateral preoptic area; anterior, lateral and dorsal hypothalamic areas), mesencephalon (SNc, VTA) and rhombencephalon (locus coeruleus, DRN) (Bruinvels et al., 1994; Sari et al., 1997, 1999; Riad et al., 2000; Makarenko et al., 2002).

The 5-HT_{2A} and the 5-HT_{2C} receptors have striking amino acid homology and their actions are mediated by the activation of phospholipase C, with a resulting depolarization of the host cell (Cooper et al., 1996; Stanford, 2001). Receptors of the 5-HT₂ subfamily are located within postsynaptic structures, predominantly on proximal and distal dendritic shafts. 5-HT_{2A} and 5-HT_{2C} receptors have been demonstrated in the cerebral cortex, limbic system (septal nuclei, hippocampal formation, amygdala), basal forebrain (nucleus of the diagonal band of Broca, bed nucleus of the stria terminalis, ventral pallidum), basal ganglia (nucleus accumbens, caudate-putamen), thalamus, hypothalamus (medial and lateral preoptic areas, ventromedial nucleus, mammillary nucleus), mesencephalon (SNc, VTA, VPAG) and rhombencephalon (LDT/PPT, DRN, MRN, locus coeruleus, mPRF) (Mengod et al., 1990; Pompeiano et al., 1994; Cornea-Hébert et al., 1999; Clemett et al., 2000; Nichols, 2004).

The 5-HT₃ receptor consists of two subtypes whose heteromeric combination seems necessary to provide its full functional features (Dubin et al., 1999; Hanna et al., 2000; Yakel, 2000). The 5-HT₃ receptor is present in cortical and subcortical structures. The latter include the hippocampus, amygdala, lateral septal nucleus, accumbens nucleus, caudate-putamen, VTA, VPAG and DRN (Kilpatrick et al., 1987, 1988; Laporte et al., 1992; Morales et al., 1998).

The 5-HT₇ receptor, is part of the G-protein superfamily of receptors, which contain seven transmembrane regions and its stimulation leads

to an increase in cAMP production (Thomas and Hagan, 2004). The 5-HT₇ receptor is expressed in a number of telencephalic (cerebral cortex, olfactory system, limbic system, basal forebrain, basal ganglia), diencephalic (thalamus, hypothalamus), mesencephalic (substantia nigra pars reticulata, central grey) and rhombencephalic (DRN, MRN, locus coeruleus) areas (To et al., 1995; Gustafson et al., 1996; Neumaier et al., 2001).

Influence of serotonergic afferents to the VTA and the SNc on the firing rate and bursting activity of DA neurons and DA release

Activation of the 5-HT_{1A} (postsynaptic) and the 5-HT_{2C} receptors tends to inhibit the firing rate and neurotransmitter release of DA neurons in the VTA and the SNc. On the other hand, activation of the 5-HT_{1B} and the 5-HT₃ receptors induces the opposite effects (Monti and Monti, 2007). It should be stressed that either direct or indirect mechanisms can be involved in the effect of 5-HT receptor activation on the functional activity of DA neurons.

Role of dopamine in the modulation of the behavioural state

The data pertinent to the role of DA in the regulation of sleep variables were obtained mainly from (1) dopamine transporter (DAT) knockout mice; (2) animals with neurotoxin-provoked cell loss in the SNc, the VTA and the VPAG and (3) pharmacological studies, in which selective and relatively selective DA receptor agonists and antagonists, were administered to laboratory animals and man.

DA transporter knockout mice

Wisor et al. (2001) quantified sleep and W in DAT knockout homozygote and heterozygote mice compared with wild-type littermates. Homozygous mice showed a significant increase of wake time and a reduction of non-REM sleep (NREMS) during the light phase compared with heterozygous

or wild-type littermates. The increment of W in the homozygous mice was not necessarily dependant on the increase of locomotor activity because it occurred under undisturbed baseline conditions and even in the absence of locomotor activity (Wisor et al., 2001).

Neurotoxin-provoked cell loss in the VTA, the SNc and the VPAG

Systemic or intracerebral administration of MPTP, a neurotoxin chemically related to heroin, produces cell loss in monoaminergic nuclei in the brainstem of laboratory animals and a syndrome similar to PD (Langston et al., 1984). Although MPTP was found to have a marked preferential neurotoxic effect on DA-containing neurons of the SNc and the VTA, it also affects serotonergic and noradrenergic cells (Takada et al., 1987). Systemic injection of a single dose (2 mg/kg) of MPTP selectively suppressed REMS for 2.5–3.5 h in the cat. On the other hand, daily injection of 5 mg/kg of MPTP for five consecutive days induced a reduction of REMS that lasted 6–9 days after the last dose (Pungor et al., 1990). In contrast, injection of 2.5–25 mg/kg MPTP induced a dose-dependant decrease of both REMS and NREMS in the rat (Lelkes et al., 1991). The immediate onset of REMS suppression after MPTP could be tentatively ascribed to the simultaneous release of DA, noradrenaline and 5-HT from the lesioned cells, and the inhibition of cholinergic and glutamatergic neurons in the brainstem responsible for the induction and maintenance of the behavioural state.

Lu et al. (2006) tested the effect of lesions of the VPAG with 6-hydroxydopamine or ibotenic acid on sleep and W in the rat. 6-Hydroxydopamine lesioned 55–65% of the tyroxine hydroxylase-immunoreactive neurons of the VPAG and significantly increased NREMS and REMS whereas W was reduced. Microinjection of ibotenic acid into the VPAG lesioned up to 85% of the tyroxine hydroxylase-immunoreactive cells. NREMS and REMS also showed a significant increase. Of note, REMS was augmented predominantly during the light phase of the light–dark cycle.

Administration of selective and relatively selective DA receptor agonists and antagonists

Systemic administration of the selective DA D₁ receptor agonist SKF 38393 induces desynchronization of the EEG and behavioural arousal in both rabbits and rats. This electroencephalographic pattern is associated with grooming or attentive posture in the rat. On the other hand, the selective DA D₁ receptor antagonist SCH 23390 tends to produce sedation in the monkey and the rabbit (Ongini et al., 1985; Bo et al., 1988). The effect of SKF 38393 on behavioural and EEG arousal is prevented by pretreatment with SCH 23390, whereas the DA D₂ receptor antagonist sulpiride is ineffective in this respect (Bo et al., 1988) (Table 1).

The actions of systemic SKF 38393 or SCH 23390 on sleep and W have been characterised in rats prepared for chronic polysomnographic recordings. SKF 38393 (0.1–10 mg/kg) significantly increases W and reduces SWS and REMS. In contrast, SCH 23390 (0.003–2 mg/kg) dose-dependantly reduces W and increases SWS and REMS. Pretreatment with SCH 23390 prevents the effect of the DA D₁ agonist on NREMS and W (Monti et al., 1990a; Trampus and Ongini, 1990). Similarly as does SKF 38393, the DA D₁ receptor agonist A 68930 (0.003–0.3 mg/kg) increases W and spontaneous grooming and reduces NREMS and REMS in the rat. SCH 23390 (0.003 mg/kg) partly prevents the effect of A 68930 on W and NREMS (Trampus et al., 1993).

Eder et al. (2003) characterised the effect of the DA receptor antagonist NNC-687, a benzazepine derivative with high affinity for the D₁ receptor, on sleep variables in healthy young men. The D₁ antagonist (5–15 mg) increased the length of the first NREM period, the frequency of delta EEG waves and the mean burst duration of sleep spindles. In contrast, W and REMS remained unchanged.

How can the effects of systemic administration of DA D₁ receptor agonists on sleep and W be understood? As was mentioned earlier, the DA D₁ receptor is coupled to adenylate cyclase, and its stimulation depolarises neurons ascending to the thalamus, the lateral hypothalamus and the basal forebrain, and cells descending to the DRN and

Table 1. The effects of DA D₁, D₂-preferring, or D₃-preferring receptor agonists on sleep and waking in the rat

Compound	W	SWS	REMS	Behaviour
SKF 38393 (D ₁ agonist)	+	–	–	Grooming
Talipexole (D ₂ presynaptic)	–	+	+	Hypomotility
Apomorphine (D ₁ >D ₂ agonist)				
Presynaptic	–	+	+	Hypomotility and sedation
Postsynaptic	+	–	–	Hyperactivity and stereotyped responses
Bromocriptine (D ₂ >D ₁ agonist)				
Presynaptic	–	+	+	Hypomotility
Postsynaptic	+	–	–	Hyperactivity
Pergolide (D ₂ >D ₁ agonist)				
Presynaptic	–	+	–	Hypomotility
Postsynaptic	+	–	–	Hyperactivity
Quinpirole (D ₂ >D ₃ agonist)				
Presynaptic	–	n.s.	n.s.	No change
Postsynaptic	+	n.s.	n.s.	Hyperactivity
Pramipexole (D ₃ >D ₂ agonist)				
Presynaptic	–	+	+	Hypomotility
Postsynaptic	+	–	–	Hyperactivity

Abbreviations: W, waking; SWS, slow wave sleep; REMS, REM sleep; n.s., non-significant; +, increased; –, decreased.

the locus coeruleus. Some of these neuronal groups project to the non-specific thalamocortical projection system, where they stimulate cortical activation. Some other cell groups project through the ventral extrathalamic pathway and stimulate cortical activation by excitatory influences on the cerebral cortex (Jones, 2005).

Systemic (0.5–32 mg/kg), i.c.v. (0.1–1 nmol) or intra-accumbens (5–80 µg) administration of (–)3-PPP, a selective DA D₂ autoreceptor agonist, suppresses locomotor activity and induces behavioural and electrocorticographic sleep in the rat. The intra-accumbens-induced suppression of locomotor activity is prevented by the systemic administration of a small selectively presynaptic D₂-blocking dose of the DA D₂ antagonist haloperidol (25–50 µg/kg) (Bagetta et al., 1987). Similar behavioural effects were observed after systemic injection of the D₂ autoreceptor agonist talipexole (0.05–0.2 mg/kg) in the rat. In addition, the compound increased the electroencephalographic spectral power in the low-frequency bands (Kropf and Kuschinsky, 1991).

The effects of systemic administration of the DA D₂ receptor agonists apomorphine (D₁>D₂),

bromocriptine (D₂>D₁), pergolide (D₂>D₁) or quinpirole (D₂>D₃) have been compared with those produced by the D₂ antagonists haloperidol or YM-09151-2. Apomorphine (0.025–2 mg/kg) or bromocriptine (0.25–6 mg/kg) produce biphasic effects, such that low doses decrease W and augment SWS and REMS whereas large doses induce the opposite effects in the rat. The actions of pergolide (0.05–0.5 mg/kg) on W and SWS are biphasic also, whereas REMS is suppressed irrespective of the amount of drug given (Monti et al., 1988). A small dose of quinpirole (0.015 mg/kg) decreases W and tends to increase SWS and REMS, whereas a large dose of the compound (1 mg/kg) increases W and reduces SWS and REMS in the rat (Monti et al., 1989). Similar wake-promoting actions accompanied by a modest increase in measures of locomotion were described following the i.c.v. infusion of a relatively large dose of quinpirole (Issac and Berridge, 2003).

Interestingly, apomorphine given bilaterally into the VTA produces behavioural and electrocorticographic sleep in a dose-dependant fashion in the rat. The effects of apomorphine are prevented by a previous microinjection into the same site of a

small selectively presynaptic dose of the DA D₂ receptor antagonist sulpiride (Bagetta et al., 1988).

A relatively large dose of haloperidol (0.02–1 mg/kg) has been shown to reduce W and to increase NREMS and the power density in the low-frequency bands in the rat (Monti et al., 1988; Ongini et al., 1993; Sebban et al., 1999). On the other hand, YM-09151-2 increases light sleep and suppresses REMS (Monti et al., 1989). Haloperidol dose-dependantly prevents the effects of apomorphine, bromocriptine or pergolide on sleep and W in the rat (Monti et al., 1988). A similar outcome was described after administration of quinpirole to rats pretreated with YM-09151-2 (Monti et al., 1989).

Thus the available evidence tends to indicate that the increase of sleep after systemic injection of a relatively small dose of a DA D₂ receptor agonist is related to the activation of D₂ autoreceptors, whereas the increase of W after the administration of a large dose of the DA ligand depends upon the activation of postsynaptic D₂ receptors.

The DA D₂ receptor is coupled to the inhibition of adenylate cyclase and its stimulation induces the hyperpolarization of the corresponding neurons. It has been proposed that the increase of W after the administration of a DA D₂ receptor agonist depends upon the indirect activation of DA D₁ receptors (Trampus and Ongini, 1990). However, it should be mentioned that DA D₂ receptors are expressed by GABAergic neurons that synapse with cells located in structures involved in the occurrence of W, including the basal forebrain and the cerebral cortex. In this respect, Seamans et al. (2001) studied the effects of DA on GABAergic inputs to prefrontal pyramidal neurons using whole-cell patch-clamp recordings in vitro. In most circumstances DA D₂ agonists reduced the postsynaptic response to a GABA_A agonist. In addition, Haj-Dahmane (2001) and Aman et al. (2007) have shown that DA D₂-like receptor agonists depolarise DRN 5-HT neurons through the activation of non-selective cationic conductance.

Thus it could be tentatively proposed that (1) stimulation of DA D₂ receptors expressed by GABAergic cells would induce their inhibition; this would lead to the disinhibition of cholinergic and glutamatergic neurons of the arousal systems

and the occurrence of cortical activation and (2) DA D₂-like receptor activation of DRN 5-HT neurons would contribute to the wake-promoting effect of DA D₂ agonists.

Quantitation of sleep variables after s.c. injection of the D₃-preferring DA agonist pramipexole has shown dose-related effects on W, SWS and REMS in the rat. Administration of a relatively small dose of the DA agonist (30 µg/kg) induces an increase of SWS and REMS whereas W is reduced. On the other hand, a greater dose of pramipexole (0.5 mg/kg) induces the opposite effects (Lagos et al., 1998). The D₂ receptor antagonist YM-09151-2 (0.5–1 mg/kg) effectively antagonises the increase of W and the reduction of SWS induced by the large dose of pramipexole, which thus suggests that the effect is related to the activation of postsynaptic D₂ receptors (Lagos et al., 1998).

Recently, the effect of the selective DA D₄ receptor antagonist L-741-741 on sleep variables was characterised in the rat. Systemic administration of L-741-741 induced dose-dependant effects on sleep and W, such that a relatively small dose (1.5 mg/kg) increased light SWS whereas a greater dose (6 mg/kg) reduced total sleep time and increased W and REM latency (Cavas and Navarro, 2006). Further studies with selective DA D₄ receptor agonists are warranted to appropriately characterise the role of the D₄ receptor in the modulation of sleep and W.

Role of serotonin in the modulation of the behavioural state

Serotonin shares with other neurotransmitters the ability to promote W and to suppress REMS. As mentioned earlier, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A/2C}, 5-HT₃ and 5-HT₇ receptors are expressed in structures that are relevant to the modulation of sleep and W, including the cerebral cortex, hippocampus, thalamus, locus coeruleus and DRN. In addition, 5-HT_{1B}, 5-HT_{2A/2C} and 5-HT₇ receptors have been characterised in the preoptic area, anterior and lateral hypothalamic areas and tuberomammillary nucleus, whereas 5-HT_{1A} and 5-HT_{2A/2C} receptors have been found in the LDT/PPT and the mPRF.

The 5-HT_{1A} receptor and sleep variables

Administration of full agonists at pre- and postsynaptic 5-HT_{1A} receptors

Systemic injection of flesinoxan or 8-OH-DPAT increases W and reduces SWS and REMS in the rat (Dugovic and Wauquier, 1987; Monti et al., 1990b, 1994; Monti and Jantos, 1992; Monti and Jantos, 2003). Pretreatment with the mixed β -adrenoceptor and 5-HT_{1A/1B} receptor antagonist (–)pindolol or the selective 5-HT_{1A} receptor antagonist *p*-MPPI reverses the effect of 8-OH-DPAT on W and SWS (Monti and Jantos, 1992; Sorensen et al., 2001). All these findings tend to indicate that the postsynaptic 5-HT_{1A} receptor has a role in the occurrence of arousal. The reduction of REMS after the administration of flesinoxan or 8-OH-DPAT could be related to the activation of postsynaptic 5-HT_{1A} receptors on REM-on neurons of the LDT/PPT.

Microdialysis perfusion or direct infusion of either 8-OH-DPAT or flesinoxan into the DRN significantly increased REMS in rats; this effect was prevented by local infusion of the 5-HT_{1A} receptor antagonist WAY 100635 (Portas et al., 1996; Monti et al., 2002). In contrast, microinjection of (–)pindolol, WAY 100635 or *p*-MPPI into the DRN reduced REMS (Monti et al., 2000, 2002; Sorensen et al., 2001). In agreement with the reciprocal interaction model proposed by McCarley and Hobson (1975), the inhibition of DRN activity following somatodendritic 5-HT_{1A} receptor stimulation suppressed the 5-HT inhibition of mesopontine cholinergic neurons and increased REMS.

Microinjection of 8-OH-DPAT or flesinoxan into the LDT or the mPRF, where structures that act to promote and to induce REMS are located, selectively inhibited REMS in the cat and the rat (Sanford et al., 1994; Horner et al., 1997; Monti and Jantos, 2003). In contrast, direct infusion of WAY 100635 into the LDT increased REMS (Monti and Jantos, 2004).

The inhibitory effect of postsynaptic 5-HT_{1A} receptors on REMS occurrence is further supported by studies carried out in mutant mice that do not express this receptor subtype. Accordingly, REMS was significantly increased during both the light and the dark phases of the light/dark cycle in

5-HT_{1A} knockout mice compared with wild-type animals. In addition, systemic 8-OH-DPAT had no effect on sleep or W in these mutant mice (Boutrel et al., 2002) (Table 2).

Administration of full agonists at 5-HT_{1A} autoreceptors and partial agonists at postsynaptic 5-HT_{1A} receptors

Buspirone, ipsapirone or gepirone have been shown to increase the time awake and to reduce SWS and REMS when given systemically to rats, and the effect persists in 5,7-dihydroxytryptamine-pretreated animals (Lerman et al., 1986; Monti et al., 1995a). Similar sleep-disrupting effects of buspirone or ipsapirone have been described in human subjects with normal sleep and in patients with insomnia (Seidel et al., 1985; Manfredi et al., 1991). The decrease of REMS after administration of the azapirones is compatible with the reciprocal interaction hypothesis of the regulation of desynchronised sleep. Accordingly, the azapirones would be acting on postsynaptic 5-HT_{1A} receptors in the LDT/PPT to inhibit REM-on neurons.

Administration of selective serotonin reuptake inhibitors

The effects of acute administration of selective serotonin reuptake inhibitors (SSRIs) on sleep have been studied in laboratory animals, healthy adults and depressed patients (Armitage, 1996; Staner et al., 1999; Oberndorfer et al., 2000). SSRIs are potent REMS suppressors, prolonging the latency to the first REM period. Direct infusion of fluoxetine into the DRN induced a significant increment of REMS in the rat. In contrast, microinjection of fluoxetine into the LDT or the mPRF produced the opposite effect; and pretreatment with WAY 100635 prevented the reduction of REMS (Monti and Jantos, 2005).

Monaca et al. (2003) examined the effect of the SSRI citalopram on REMS in 5-HT_{1A} or 5-HT_{1B} knockout mice. Citalopram suppressed REMS in wild-type and 5-HT_{1B}^{–/–} mice but not in 5-HT_{1A}^{–/–} mutants. The 5-HT_{1A} antagonist WAY 100635 prevented the citalopram-induced inhibition of REMS in the wild-type and the 5-HT_{1B} knockout

Table 2. The effects of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A/2C}, or 5-HT₃ receptor agonists on sleep and waking in the rat

Compound	W	SWS	REMS
<i>8-OH-DPAT, flesinoxan</i> (5-HT _{1A} agonists)			
Somatodendritic (Microinjection into the DRN)	n.s.	n.s.	+
Postsynaptic (Systemic injection)	+	—	—
<i>Buspirone, ipsapirone, gepirone</i> (Partial agonists at postsynaptic sites)			
(Systemic injection)	+	—	—
<i>Fluoxetine</i> (Selective serotonin reuptake inhibitor)			
Somatodendritic (Microinjection into the DRN)	n.s.	n.s.	+
Postsynaptic (Systemic injection)	n.s.	n.s.	—
<i>CGS 12066B; CP-94,253</i> (5-HT _{1B} agonists)			
(Systemic injection)	+	—	—
<i>DOI</i> (5-HT _{2A/2C} agonist)			
(Microinjection into the DRN)	n.s.	n.s.	—
(Systemic injection)	+	—	—
<i>m-chlorophenylbiguanide</i> (5-HT ₃ agonist)			
(i.c.v. injection)	+	—	—

Abbreviations: DRN, dorsal raphe nucleus; W, waking; SWS, slow wave sleep; REMS, REM sleep; n.s., non-significant; +, increased; —, decreased.

mice. However, pretreatment with the 5-HT_{1B} antagonist GR 127935 was ineffective in this respect. It was concluded that the action of citalopram on REMS depends exclusively on the activation of 5-HT_{1A} receptors. However, there is conclusive evidence showing that administration of selective 5-HT_{1B} agonists suppresses REMS in the rat, which is discussed in the next section.

The 5-HT_{1B} receptor and sleep variables

Full agonists at postsynaptic 5-HT_{1B} receptors

Few studies have been published on the effect of 5-HT_{1B} receptor ligands on sleep variables. Systemic administration of the selective 5-HT_{1B} receptor agonists CGS 12066B or CP-94,253 significantly increased W and reduced SWS and REMS in the rat (Bjorvatn and Ursin, 1994; Monti

et al., 1995b). The mixed β -adrenoceptor/5-HT_{1A/1B} receptor antagonist pindolol prevented the increase of W and reduction of SWS by CP-94,253. However, pindolol failed to prevent the suppression of REMS (Monti et al., 1995b).

Quantization of spontaneous sleep-waking cycles in 5-HT_{1B} receptor knockout mice has shown that REMS is increased whereas SWS is reduced during the light phase (Boutrel et al., 1999). Thus, the limited available evidence indicates that 5-HT_{1B} receptor activation facilitates the occurrence of W and negatively influences REMS.

The 5-HT_{2A/2B/2C} receptors

5-HT₂ receptor agonists

Systemic administration of the 5-HT_{2A/2C} receptor agonists DOI or DOM has been shown to reduce

SWS and REMS and to augment W in the rat (Dugovic and Wauquier, 1987; Dugovic et al., 1989; Monti et al., 1990b). The i.p. and p.o. administration of the selective 5-HT_{2C} receptor agonists RO 60-0175/ORG 35030 or RO 60-0332/ORG 35035 also induced an increase of W and a reduction of REMS in the rat (Martin et al., 1998).

5-HT₂ receptor antagonists

Injection of the 5-HT_{2A/2C} receptor antagonists ritanserin, ketanserin, ICI 170,809 or sertindole at the beginning of the light period induced a significant increase of SWS and a reduction of REMS in the rat. Waking was also diminished in most studies (Dugovic et al., 1989; Tortella et al., 1989; Monti et al., 1990b; Silhol et al., 1991; Coenen et al., 1995; Kirov and Moyanova, 1998).

More recently, the action of subtype-selective 5-HT₂ receptor antagonists on sleep variables was assessed in the rat. Systemic administration of the 5-HT_{2A} receptor antagonist EMD 281014 significantly reduced REMS (Monti and Jantos 2006b), whereas the 5-HT_{2B} antagonist SB-215505 augmented W and suppressed SWS and REMS (Kantor et al., 2004). Moreover, oral administration of the 5-HT_{2C} antagonist SB-243213 to rats significantly increased SWS and reduced REMS during the light period (Smith et al., 2002). However, REMS suppression was the only noticeable effect when the compound was given by the subcutaneous route (Monti and Jantos, 2006b).

Effect of 5-HT₂ antagonists on the DOI-induced disruption of sleep and W. Pretreatment with ritanserin prevented the enhancement of W and the deficit of SWS induced by DOI or DOM but not the REMS suppression (Dugovic et al., 1989; Monti et al., 1990a). In order to gain further insight into the roles of 5-HT_{2A} and 5-HT_{2C} receptors in the DOI-induced disruption of the sleep–wake cycle, animals were pretreated with either EMD 281014 or SB-243213, which selectively block the 5-HT_{2A} or the 5-HT_{2C} receptor respectively. EMD 281014 prevented the increase of W and the reduction of SWS induced by DOI; however, REMS remained suppressed (Monti and

Jantos, 2006b). The failure of EMD 281014 to prevent the suppression of REMS tends to indicate that the effect of DOI is not restricted to the 5-HT system. The finding that systemic DOI increases the firing rate and the burst firing of DA neurons and the release of DA in the VTA of the rat (Pehec et al., 2001; Bortolozzi et al., 2005) tends to support this proposal.

Microinjection of DOI directly into the DRN induced a significant reduction of REMS and of the number of REM periods. Following the microinjection of EMD 281014 or SB-243213 light SWS was significantly augmented. Pretreatment with EMD 281014 or SB-243213 antagonised the DOI-induced decrease of REMS, which indicates that it was mediated by the 5-HT_{2A/2C} receptors located in the DRN (Monti and Jantos, 2006c).

The 5-HT₃ receptor

Not much is known about the role of the 5-HT₃ receptor in the regulation of sleep and W. Injection of the 5-HT₃ receptor agonist *m*-chlorophenylbiguanide into the left lateral ventricle increased W and REMS latency, whereas SWS, REMS and the number of REM periods were reduced in the rat (Ponzoni et al., 1993). In contrast, systemic administration of the 5-HT₃ antagonists MDL 72222 or ondansetron significantly increased SWS and/or REMS respectively (Tissier et al., 1990; Adrien et al., 1992; Ponzoni et al., 1993). Moreover, pretreatment with MDL 72222 prevented the increase of W and reduction of SWS and REMS induced by *m*-chlorophenylbiguanide (Ponzoni et al., 1993). Concerning the mechanism underlying the *m*-chlorophenylbiguanide-induced disruption of the sleep–wake cycle, it has been proposed that 5-HT₃ receptor agonists presumably act by increasing the release of several endogenous neurotransmitters (Kilpatrick and Tyers, 1992), which during a second step would augment W and diminish sleep. In this respect, it was found that microinjection of *m*-chlorophenylbiguanide into the nucleus accumbens increased W and reduced SWS in the rat. The effect of the 5-HT₃ agonist was markedly attenuated in 6-hydroxydopamine-treated animals and was antagonised by MDL 72222 and the DA D₁ or D₂ receptor

antagonists SCH 23390 or YM-09151-2 respectively (Ponzoni et al., 1995; Monti et al., 1999). Thus, presently available evidence supports the proposal that the increase of W and reduction of SWS after 5-HT₃ receptor activation is related, at least in part, to the increased availability of DA at central sites.

The 5-HT₇ receptor

5-HT₇ receptor antagonists

SB-269970 and SB-656104 were recently reported to be potent 5-HT₇ receptor antagonists (Hagan et al., 2000; Forbes et al., 2002). Systemic administration of either 5-HT₇ receptor ligand to rats at the beginning of the light phase has been shown to reduce the total amount of REMS and to increase REMS latency. Values of W and SWS were not significantly modified (Hagan et al., 2000; Thomas et al., 2003). Hedlund et al. (2005) have established that 5-HT₇ receptor knockout mice spend less time in REMS during the light phase compared with their wild-type counterparts. On the other hand, there is no difference between the genotypes in time spent in W or SWS. Infusion of SB-269970 directly into the DRN also induced a suppression of REMS in the rat (Monti and Jantos, 2006a).

Glass et al. (2003) and Roberts et al. (2004) have proposed, on the basis of a series of functional studies, that 5-HT₇ receptors in the DRN are localised on GABAergic cells and terminals. Thus it is conceivable that microinjection of SB-269970 into the DRN reduces GABAergic inhibition of 5-HT neurons and increases 5-HT release at postsynaptic sites, including the LDT/PPT and the mPRF, with the resultant suppression of REMS. To test this hypothesis, muscimol was microinjected into the DRN prior to the administration of the 5-HT₇ antagonist. It was observed that the GABA_A receptor agonist prevented the SB-269970-induced decrease of REMS (Monti and Jantos, 2006a). Thus, knockout mice strains that lack the 5-HT₇ receptor and rats given a 5-HT₇ receptor antagonist spend less time in REMS, which might be partly related to a reduction of the

inhibitory effect of GABA on DRN 5-HT neurons.

Clinical context

Sleep in patients with Parkinson's disease

Nocturnal sleep is frequently disrupted in patients with PD. Levodopa, DA agonists, anticholinergic medications and other drugs used for PD may indirectly improve or worsen sleep by changing motor symptoms or promoting W (Schäffer and Greulich, 2000). Excessive daytime sleepiness (EDS) is also a common problem in PD; it limits the symptomatic treatment and further compromises the patient's quality of life. EDS has been tentatively ascribed to a DA-deficiency state in the brain resulting from the degeneration of DA neurons and the loss of a facilitatory effect on neural structures relevant for the promotion of W (Homann et al., 2002). Sudden and irresistible sleep attacks have also been reported in patients with PD on levodopa or DA agonists. The latter include ergot agonists (bromocriptine, cabergoline, lisuride, pergolide) and non-ergot agonists (pramipexole, ropinirole) (Wenzel et al., 2002). It has been proposed that DA agonists induce sleep attacks by down-regulating DA input to W-promoting systems in the brainstem, hypothalamus and basal forebrain. This effect is explained as being dependant on the stimulation of presynaptic DA receptors (Frucht et al., 2000). However, further studies are needed to resolve this issue.

Sleep in schizophrenia patients

Insomnia is a common feature in schizophrenia. However, it seldom is the predominant complaint. Nevertheless, severe insomnia is often seen during exacerbations of schizophrenia and may actually precede the appearance of other symptoms of relapse. The sleep disturbances of either never-medicated or previously treated schizophrenia patients are characterised by a sleep-onset and maintenance insomnia. In addition, stage 4 sleep, SWS (stages 3 and 4), and REM latency are frequently decreased. On the other hand, REMS in

minutes is comparable between schizophrenia patients and controls in most studies. Concerning the mechanisms involved in the disruption of sleep in schizophrenia patients, it has been proposed that they could be related to an overactivity of the dopaminergic system, dependant on the increased density of D₂ receptors and the enhanced sensitivity of dopaminergic neurotransmission (Laruelle et al., 1996; Abi-Dargham et al. 1998). However, the possibility remains that insomnia in schizophrenia patients is not exclusively DA dependant but that other neurotransmitter systems are involved in the disruption of this behavioural state (Monti and Monti, 2004).

Polysomnographic and clinical studies tend to indicate that the atypical antipsychotics olanzapine, clozapine, risperidone, quetiapine and ziprasidone increase total sleep and stage 2 sleep. Moreover, olanzapine and risperidone enhance SWS whereas quetiapine and ziprasidone reduce REMS (Touyz et al., 1978; Salin-Pascual et al., 1999; Yamashita et al., 2002; Cohrs et al., 2004, 2005; Monti and Monti, 2004; DeMartinis and Winokur, 2006).

Atypical antipsychotic drugs bind to a wide variety of central nervous system receptors. They produce their effects by blocking DA (D₁, D₂, D₃, D₄), 5-HT (5-HT_{2A}, 5-HT_{2C}, 5-HT₆, 5-HT₇), α -adrenergic (α_1 , α_2), histamine (H₁) and acetylcholine (muscarinic) receptors.

The blockade of DA D₂ receptors could be partly responsible for the improvement of sleep in schizophrenia patients (Monti and Monti, 2004). However, the amelioration of sleep has been related almost exclusively to the blockade of α_1 , H₁ and cholinergic (muscarinic) receptors. This is based on the premise that antagonists of α_1 receptors, first-generation H₁ receptors or cholinergic (muscarinic) receptors produce somnolence, an increased likelihood of falling asleep and reduced concentration (Monti, 1987; Heller-Brown and Tylor, 1996; Monti and Monti, 2000). It should be mentioned that the increase of stage 4 sleep and SWS induced by olanzapine or risperidone in schizophrenia patients could be related to the selective blockade of 5-HT_{2A/2C} receptors (Idzikowski et al., 1987; Sharpley et al., 1994).

Conclusions

Dopamine in the regulation of sleep and waking

Although many questions remain about the role of DA in regulating sleep and W, recent genetic, electrophysiological and neuropharmacological studies have revealed much detailed information about this process. However, attempts to characterise the role of DA receptors on sleep variables have been limited in a great measure, to investigations of the DA D₁ and D₂ receptors. Most studies have examined the effects of systemic administration of selective and relatively selective agonists and antagonists on sleep and W in the rat.

Behavioural arousal is impaired in DA D₁ receptor knockout mice. On the other hand, systemic administration of a selective DA D₁ agonist induces desynchronization of the electroencephalogram and behavioural arousal, together with an increase of W and a reduction of SWS and REMS. In contrast, injection of a DA D₁ antagonist tends to produce sedation and to reduce W, whereas SWS and REMS are augmented.

Mice with genetically induced lesions that target the D₂ receptor show reduced levels of spontaneous locomotor activity. Similar effects are observed following systemic, i.c.v. or intra-accumbens injection of a selective DA D₂ autoreceptor agonist. Systemic administration of DA D₂ agonists induces biphasic effects, such that low doses reduce W and increase SWS and REMS whereas large doses induce the opposite effects. Drugs with DA D₂ receptor blocking-properties increase NREMS and reduce W. In addition, they prevent the effect of DA D₂ agonists on sleep and W.

D₃ receptor knockout mice display increased locomotor activity. Furthermore, administration of a small dose of a DA D₃-preferring agonist induces somnolence and sleep in laboratory animals and man.

The process by which the activation of DA D₁ and D₂ receptors facilitates the state of W is not fully understood. DA neurons of the VTA and the SNc do not change their mean firing rates across the sleep-wake cycle. Under physiological conditions DA-containing neurons in the midbrain fire

in one of the two patterns: slow, irregular, spontaneous action potentials or bursts of spikes that show a progressive decrease in amplitude and increase in duration. The burst firing is modulated by afferent inputs from several structures relevant for the regulation of the behavioural state, including the LDT/PPT, the DRN, the locus coeruleus and the lateral hypothalamus. Presently available evidence indicates that activation of glutamatergic (NMDA, AMPA, kainate), cholinergic (nicotinic, muscarinic), serotonergic [5-HT_{1A} (somatodendritic), 5-HT₃] or orexinergic (A, B) receptors increases burst firing and DA release by VTA and SNc neurons. In contrast, the activation of serotonergic 5-HT_{1A} (postsynaptic) and 5-HT_{2C} receptors or the blockade of noradrenergic α_1 -adrenoceptors reduces the occurrence of burst firing and of neurotransmitter release. Thus DA neurons in the midbrain would show a change in temporal pattern rather than firing rate during the sleep–wake cycle.

Serotonin in the regulation of sleep and waking

Attempts to characterise the roles of 5-HT receptors on sleep variables have been limited to studies of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A/2B/2C}, 5-HT₃ and 5-HT₇ receptors. Early studies examined the effects of predominantly systemic administration of selective and relatively selective agonists and antagonists on sleep and W in the rat and the cat. More recently, results from several studies have quantified the spontaneous sleep/waking cycles in serotonin 5-HT_{1A}, 5-HT_{1B} or 5-HT₇ receptor knockout mice. Much less is known about the effects of local microinjection of 5-HT receptor ligands into central nervous system structures relevant to the regulation of sleep and W, including the DRN, the LDT/PPT and the mPRF. All 5-HT receptor agonists studied to date share the ability to promote W and to suppress REMS when given by the i.c.v., i.p. or s.c. route.

Although the process by which the activation of 5-HT_{1A} and 5-HT_{1B} receptors facilitates the state of W is still unknown, it should be mentioned that many GABAergic cells in the basal forebrain, hippocampus and neocortex on which 5-HT_{1A} and 5-HT_{1B} receptors are expressed are hyperpolarised

by serotonin released from the DRN (Parnavelas, 1990; Araneda and Andrade, 1991; Detari et al., 1999; Newberry et al., 1999). Thus the inhibition of GABAergic neurons following the activation of 5-HT_{1A} and 5-HT_{1B} receptors could account, at least in part, for their facilitatory effect on W. The activation of 5-HT_{1A} and 5-HT_{1B} receptors may attenuate GABAergic input and thereby indirectly increase the release of acetylcholine and glutamate at cortical and subcortical sites. However, 5-HT_{1A} and 5-HT_{1B} receptor-dependant inhibition of cholinergic and glutamatergic neurons in the LDT/PPT and the mPRF, respectively, would be directly responsible for REMS suppression.

Activation of 5-HT_{2A} and 5-HT_{2C} receptors enhances the release of acetylcholine in the medial prefrontal cortex and the hippocampus, and of DA in the medial prefrontal cortex of the rat (Alex and Pehek, 2007). Thus, the increased availability of DA and acetylcholine at central sites after 5-HT_{2A/2C} receptor activation could be responsible, at least in part, for the increased incidence of W. Mesopontine cholinergic cells do not express 5-HT_{2A/2C} receptors. The LDT/PPT neurons that express these receptors are mainly inhibitory GABAergic interneurons. Accordingly, the suppression of REMS after the stimulation of 5-HT_{2A/2C} receptors can be related to the activation of the GABAergic interneurons located within and around the LDT/PPT that express those receptors.

Not much is known about the mechanisms subserving the increase of W and the reduction of REMS after activation of the 5-HT₃ receptor. This receptor is well known for stimulating the release of DA, acetylcholine, noradrenaline, 5-HT, GABA and glutamate in the brainstem, the limbic system, the basal forebrain and the cortex, which can tentatively explain the disruption of sleep variables. However, further studies are needed to resolve this issue.

Almost no data are available on DA-serotonin interaction in the regulation of sleep and W. Notwithstanding the above, it has been shown that VTA and SNc DA neurons and DRN 5-HT neurons influence each other. Thus, depending on the receptor subtype involved 5-HT either facilitates or inhibits the burst firing and DA

release of DA cells (Alex and Pehek, 2007). On the other hand, activation of DA D₂-like receptors tends to increase the activity of DRN 5-HT cells (Haj-Dahmane, 2001; Aman et al., 2007). Thus it can be speculated that microinjection of DA and 5-HT receptor ligands into the DRN and the VTA/SNc, respectively, would affect the actions of the corresponding neurons on sleep and W.

Abbreviations

DA	dopamine
DAT	dopamine transporter
DRN	dorsal raphe nucleus
EDS	excessive daytime sleepiness
GABA	γ -aminobutyric acid
5-HT	serotonin
LDT/PPT	laterodorsal and pedunculopontine tegmental nuclei
mPRF	medial pontine reticular formation
MRN	median raphe nucleus
NREMS	non-REM sleep
PD	Parkinson's disease
REMS	rapid-eye-movement sleep
SNc	substantia nigra pars compacta
SSRIs	selective serotonin reuptake inhibitors
SWS	slow wave sleep
VPAG	ventral periaqueductal grey matter
VTA	ventral tegmental area
W	waking

References

- Abi-Dargham, A., Gil, R., Krystal, J., Baldwin, R.M., Seibyl, J.P., Bowers, M., van Dyck, C.H., Charney, D.S., Innis, R.B. and Laruelle, M. (1998) Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am. J. Psychiatry*, 155: 761–767.
- Adrien, J., Tissier, M.H., Lanfumey, L., Haj-Dahmane, S., Jolas, T., Franc, B. and Hamon, M. (1992) Central action of 5-HT₃ receptor ligands in the regulation of sleep-wakefulness and raphe neuronal activity in the rat. *Neuropharmacology*, 31: 519–529.
- Aghajanian, G.K. and Lakoski, J.M. (1984) Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K⁺ conductance. *Brain Res.*, 305: 181–185.
- Alam, M.N., McGinty, D. and Szymusiak, R. (1995) Neuronal discharge of preoptic/anterior hypothalamic thermosensitive neurons: relation to NREM sleep. *Am. J. Physiol.*, 269: R1240–R1249.
- Alam, M.N., Szymusiak, R., Gong, H., King, J. and McGinty, D. (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. *J. Physiol. Lond.*, 521: 679–690.
- Alex, K.D. and Pehek, E.A. (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacol. Ther.*, 113: 296–320.
- Aman, T.K., Shen, R.Y. and Haj-Dahmane, S. (2007) D₂-like dopamine receptors depolarize dorsal raphe serotonin neurons through the activation of nonselective cationic conductance. *J. Pharmacol. Exp. Ther.*, 320: 376–385.
- Araneda, R. and Andrade, R. (1991) 5-hydroxytryptamine₂ and 5-hydroxytryptamine_{1A} receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience*, 40: 399–404.
- Armitage, R. (1996) Effects of antidepressant treatment on sleep EEG in depression. *J. Psychopharmacol.*, 10(Suppl. 1): 22–25.
- Austin, M.C., Weikel, J.A., Arango, V. and Mann, J.J. (1994) Localization of serotonin 5-HT_{1A} receptor mRNA in neurons of the human brainstem. *Synapse*, 18: 276–279.
- Baez, M., Kursar, J.D., Helton, I.A., Wainscott, D.B. and Nelson, D.L. (1995) Molecular biology of serotonin receptors. *Obesity Res.*, 3(Suppl. 4): 441S–447S.
- Bagetta, G., Corasaniti, M.T., Strongoli, M.C., Sakurada, S. and Nistico, G. (1987) Behavioral and ECoG spectrum power effects after intraventricular microinjection of drugs altering dopaminergic transmission in rats. *Neuropharmacology*, 26: 1047–1052.
- Bagetta, G., De Sarro, G., Priolo, E. and Nistico, G. (1988) Ventral tegmental area: site through which dopamine D₂-receptor agonists evoke behavioral and electrocortical sleep in rats. *Br. J. Pharmacol.*, 95: 860–866.
- Baghdoyan, H.A. and Lydic, R. (2002) Neurotransmitters and neuromodulators regulating sleep. In: Bazil C.W., Marlow B.A. and Sammaritano M.R. (Eds.), *Sleep and Epilepsy: The Clinical Spectrum*. Elsevier, Amsterdam, pp. 17–44.
- Bjorvatn, B. and Ursin, R. (1994) Effects of the selective 5-HT_{1B} agonist, CGS 12066B, on sleep/waking stages and EEG power spectrum in rats. *J. Sleep Res.*, 3: 97–105.
- Bo, P., Ongini, E., Giorgetti, A. and Savoldi, F. (1988) Synchronization of the EEG and sedation induced by neuroleptics depend upon blockade of both D-1 and D-2 dopamine receptors. *Neuropharmacology*, 27: 799–805.
- Bortolozzi, A., Diaz-Mataix, L., Scorza, C., Celada, P. and Artigas, F. (2005) The activation of 5-HT_{2A} receptors in prefrontal cortex enhances dopaminergic activity. *J. Neurochem.*, 95: 1597–1607.
- Boutrel, B., Franc, B., Hen, R., Hamon, M. and Adrien, J. (1999) Key role of 5-HT_{1B} receptors in the regulation of paradoxical sleep as evidenced in 5-HT_{1B} knockout mice. *J. Neurosci.*, 19: 3204–3212.

- Boutrel, B., Monaca, C., Hen, R., Hamon, M. and Adrien, J. (2002) Involvement of 5-HT_{1A} receptors in homeostatic and stress-induced adaptive regulations of paradoxical sleep: studies in 5-HT_{1A} knock-out mice. *J. Neurosci.*, 22: 4686–4692.
- Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D. and Palacios, J.M. (1994) Localization of the 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} receptor messenger RNA in rodent and primate brain. *Neuropharmacology*, 33: 367–386.
- Cavas, M. and Navarro, J.F. (2006) Effects of selective dopamine D₄ receptor antagonist, L-741,741, on sleep and wakefulness in the rat. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 30: 668–678.
- Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P. and Fone, K.C.F. (2000) Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. *Neuropharmacology*, 39: 123–132.
- Coenen, A.M.L., Ates, N., Skarsfeldt, T. and Luijcklaar, E.L.J.M. (1995) Effects of sertindole on sleep-wake states, electroencephalogram, behavioral patterns, and epileptic activity in rats. *Pharmacol. Biochem. Behav.*, 51: 353–357.
- Cohrs, S., Meier, A., Neumann, A.C., Jordan, W., Ruther, E. and Rodenbeck, A. (2005) Improved sleep continuity and increased slow wave sleep and REM latency during ziprasidone treatment: a randomized, controlled, crossover trial of 12 healthy male subjects. *J. Clin. Psychiatry*, 66: 989–996.
- Cohrs, S., Rodenbeck, A., Guan, Z., Pohlmann, K., Jordan, W., Meier, A. and Ruther, E. (2004) Sleep-promoting properties of quetiapine in healthy subjects. *Psychopharmacology*, 174: 421–429.
- Cooper, J.R., Bloom, F.E. and Roth, R.H. (1996) *The Biochemical Basis of Neuropharmacology* (7th edn.). Oxford University Press, Oxford.
- Cornea-Hébert, V., Riad, M., Wu, C., Singh, S.K. and Descarries, L. (1999) Cellular and subcellular distribution of the serotonin 5-HT_{2A} receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, 409: 187–209.
- Dahan, L., Astier, B., Vautrelle, N., Urbain, N., Kocsis, B. and Chouvet, G. (2007) Prominent burst firing of dopaminergic neurons in the ventral tegmental area during paradoxical sleep. *Neuropsychopharmacology*, 32: 1232–1241.
- DeMartinis, N.A. and Winokur, A. (2006) Effects of psychiatric medications on sleep and sleep disorders. *CNS Neurol. Disord. – Drug Targets*, 6: 17–29.
- Detari, L., Rasmusson, D.D. and Semba, K. (1999) The role of basal forebrain neurons in tonic and phasic activation of the cerebral cortex. *Progr. Neurobiol.*, 58: 249–277.
- Dubin, A.E., Huvar, R., D'Andrea, M.R., Pyati, J., Zhu, J.Y., Joy, K.C., Wilson, S.J., Galindo, J.E., Glass, C.A., Luo, L., Jackson, M.R., Lovemberg, T.W. and Erlander, M.G. (1999) The pharmacological and functional characteristics of the serotonin 5HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. *J. Biol. Chem.*, 274: 30799–30810.
- Dugovic, C. and Wauquier, A. (1987) 5-HT₂ receptors could be primarily involved in the regulation of slow wave sleep in the rat. *Eur. J. Pharmacol.*, 137: 145–146.
- Dugovic, C., Wauquier, A., Leysen, J.E. and Janssen, P.A.J. (1989) Functional role of 5-HT₂ receptors in the regulation of sleep and wakefulness in the rat. *Psychopharmacology*, 97: 436–442.
- Eder, D.N., Zdravkovic, M. and Wildschiodtz, G. (2003) Selective alterations of the first NREM sleep cycle in humans by a dopamine D₁ receptor antagonist (NNC-687). *J. Psychiatry Res.*, 37: 305–312.
- Emilien, G., Maloteaux, J.-M., Geurts, M., Hoogenberg, K. and Cragg, G. (1999) Dopamine receptors – physiological understanding to therapeutic intervention potential. *Pharmacol. Ther.*, 84: 133–156.
- Fadel, J. and Deutch, A.Y. (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience*, 111: 379–387.
- Forbes, I.T., Douglas, S., Gribble, A.D., Ife, R.J., Lightfoot, A.P., Garner, A.E., Riley, G.J., Jeffrey, P., Stevens, A.J., Stean, T.O. and Thomas, D.R. (2002) SB-656104-A: a novel 5-HT₇ receptor antagonist with improved in vivo properties. *Bioorgan. Med. Chem. Lett.*, 12: 334–344.
- Frucht, S.J., Greene, P.E. and Fahn, S. (2000) Sleep episodes in Parkinson's disease: a wake-up call. *Mov. Disord.*, 15: 601–603.
- Glass, J.D., Grossman, G.H., Farnbauch, L. and DiNardo, L. (2003) Midbrain raphe modulation of nonphotic circadian clock resetting and 5-HT release in the mammalian suprachiasmatic nucleus. *J. Neurosci.*, 23: 7451–7460.
- Gonon, F.G. (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. *Neuroscience*, 24: 19–28.
- Grace, A.A. (2002) Dopamine. In: Davis K.L., Charney D., Coyle J.T. and Nemeroff C. (Eds.), *Neuropsychopharmacology – The Fifth Generation of Progress*. Lippincott Williams and Wilkins, Philadelphia, pp. 119–132.
- Grace, A.A. and Bunney, B.S. (1984) The control of firing pattern in nigral dopamine neurons: burst firing. *J. Neurosci.*, 4: 2877–2890.
- Grace, A.A. and Bunney, B.S. (1985) Low doses of apomorphine elicit two opposing influences on dopamine cell electrophysiology. *Brain Res.*, 333: 285–298.
- Gustafson, E.L., Durkin, M.M., Bard, J.A., Zgombick, J. and Branchek, T.A. (1996) A receptor autoradiographic and in situ hybridization analysis of the distribution of the 5-HT₇ receptor in rat brain. *Br. J. Pharmacol.*, 117: 657–666.
- Hagan, J.J., Price, G.W., Jeffrey, P., Deeks, N.J., Stean, T., Piper, D., Smith, M.I., Upton, N., Medhurst, A.D., Middlemiss, D.N., Riley, G.J., Lovell, P.J., Bromidge, S.M. and Thomas, D.R. (2000) Characterization of SB-269970-A, a selective 5-HT₇ receptor antagonist. *Br. J. Pharmacol.*, 130: 539–548.
- Haj-Dahmane, S. (2001) D₂-like dopamine receptor activation excites rat dorsal raphe 5-HT neurons in vitro. *Eur. J. Neurosci.*, 14: 25–34.
- Hanna, M.C., Davies, P.A., Hales, T.G. and Kirkness, E.F. (2000) Evidence for expression of heteromeric serotonin 5-HT(3) receptors in rodents. *J. Neurochem.*, 75: 240–247.

- Hedlund, P.G., Huitron-Resendiz, S., Henriksen, S.J. and Sutcliffe, J.G. (2005) 5-HT₇ receptor inhibition and inactivation-induced antidepressant like behavior and sleep pattern. *Biol. Psychiatry*, 58: 831–837.
- Heller-Brown, J. and Tylor, P. (1996) Muscarinic receptor agonists and antagonists. In: Hardman J.C. and Limbird L.E. (Eds.), *The Pharmacological Basis of Therapeutics*. McGraw-Hill, New York pp. 141–160. Chapter 7.
- Homann, C.N., Wenzel, K., Suppan, K., Ivanic, G., Kriechbaum, N., Crevenna, R. and Ott, E. (2002) Sleep attacks in patients taking dopamine agonists: review. *BMJ*, 324: 1483–1487.
- Horner, R.L., Sanford, L.D., Annis, D., Pack, A.I. and Morrison, A.R. (1997) Serotonin at the laterodorsal tegmental nucleus suppresses rapid-eye-movement sleep in freely behaving rats. *J. Neurosci.*, 17: 7541–7554.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Harting, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol. Rev.*, 46: 157–203.
- Hoyer, D. and Martin, G.R. (1996) Classification and nomenclature of 5-HT receptors: a comment on current issues. *Behav. Brain Res.*, 73: 263–268.
- Idzikowski, C., Cowen, P.J., Nutt, D. and Mills, F.J. (1987) The effects of chronic ritanserin treatment on sleep and the neuroendocrine response to l-tryptophan. *Psychopharmacology*, 93: 416–420.
- Issac, S.O. and Berridge, C.W. (2003) Wake-promoting actions of dopamine D₁ and D₂ receptor stimulation. *J. Pharmacol. Exp. Ther.*, 307: 386–394.
- Jones, B.E. (2003) Arousal systems. *Front. Biosci.*, 8: 578–586.
- Jones, B.E. (2005) From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol. Sci.*, 26: 578–586.
- Kantor, S., Jakus, R., Balogh, B., Benko, A. and Bagdy, G. (2004) Increased wakefulness and motor activity, and decreased theta activity after blockade of the 5-HT_{2B} receptor by the subtype-selective antagonist SB-215505. *Br. J. Pharmacol.*, 142: 1332–1342.
- Kia, H.K., Brisorgueil, M.J., Hamon, M., Calas, A. and Vergé, D. (1996a) Ultrastructural localization of 5-hydroxytryptamine_{1A} receptors in the rat brain. *J. Neurosci. Res.*, 46: 697–708.
- Kia, H.K., Miquel, M.C., Brisorgueil, M.J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M. and Vergé, D. (1996b) Immunocytochemical localization of 5-HT_{1A} receptor in the rat central nervous system. *J. Comp. Neurol.*, 365: 289–305.
- Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Identification and distribution of 5-HT₃ receptors in rat brain using radioligand binding. *Nature*, 330: 746–748.
- Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1988) The distribution and specific binding of the 5-HT₃ receptor ligand [³H]GR65630 in rat brain using quantitative autoradiography. *Neurosci. Lett.*, 94: 156–160.
- Kilpatrick, G.J. and Tyers, M.B. (1992) The pharmacological properties and functional roles of central 5-HT₃ receptors. In: Hamon M. (Ed.), *Central and Peripheral 5-HT₃ Receptors*. Academic Press, London, pp. 33–57.
- Kirov, R. and Moyanova, S. (1998) Age-related effects of ritanserin on the sleep-waking phases in rats. *Int. J. Neurosci.*, 93: 265–278.
- Kitai, S.T., Shepard, P.D., Callaway, J.C. and Scroggs, R. (1999) Afferent modulation of dopamine firing patterns. *Curr. Opin. Neurobiol.*, 9: 690–697.
- Kropf, W. and Kuschinsky, K. (1991) Electroencephalographic correlates of the sedative effects of dopamine agonists presumably acting on autoreceptors. *Neuropharmacology*, 30: 953–960.
- Lagos, P., Scorza, C., Monti, J.M., Jantos, H., Reyes-Parada, M., Silveira, R. and Ponzoni, A. (1998) Effects of the D₃ preferring dopamine agonist pramipexole on sleep and waking, locomotor activity and striatal dopamine release in rats. *Eur. Neuropsychopharmacol.*, 8: 113–120.
- Langston, J.W., Forno, L.S., Rebert, C.S. and Irwin, I. (1984) Selective nigral toxicity after systemic administration of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in the squirrel monkey. *Brain Res.*, 292: 390–394.
- Laporte, A.M., Koscielniak, T., Ponchant, M., Vergé, D., Hamon, M. and Gozlan, H. (1992) Quantitative autoradiographic mapping of 5-HT₃ receptors in the rat CNS using [¹²⁵I]iodo-zacopride and [³H]zacopride as radioligands. *Synapse*, 10: 271–281.
- Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., Baldwin, R.M., Seibyl, J.P., Krystal, J.H., Charney, D.S. and Innis, R.B. (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc. Natl. Acad. Sci. USA*, 93: 9235–9240.
- Lelkes, Z., Stenberg, D. and Porkka-Heiskanen, T. (1991) Effect of MPTP on sleep in rats. *Sleep Res.*, 20A: p. 154.
- Le Moal, M. and Simon, H. (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol. Rev.*, 71: 155–234.
- Lerman, J.A., Kaitin, K.I., Dement, W.C. and Peroutka, S.J. (1986) The effects of buspirone on sleep in the rat. *Neurosci. Lett.*, 72: 64–68.
- Lu, J., Zhou, T.C. and Saper, C.B. (2006) Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. *J. Neurosci.*, 26: 193–202.
- Lucas, E.A. and Serman, M.B. (1975) Effect of forebrain lesion on the polycyclic sleep-wake cycle and sleep-wake patterns in the cat. *Exp. Neurol.*, 46: 368–388.
- Luebke, J.L., Greene, R.W., Semba, K., Kamondi, A., McCarley, R.W. and Reiner, P.B. (1992) Serotonin hyperpolarizes cholinergic low threshold burst neurons in the rat laterodorsal tegmental nuclei in vitro. *Proc. Natl. Acad. Sci. USA*, 89: 743–747.
- Makerenko, I.G., Meguid, M.M. and Ugrumov, M.V. (2002) Distribution of serotonin 5-hydroxytryptamine_{1B} (5-HT_{1B}) receptors in the normal rat hypothalamus. *Neurosci. Lett.*, 328: 156–159.
- Mallick, B.N., Majumdar, S., Faisal, M., Yadav, V., Madan, V. and Pal, D. (2002) Role of norepinephrine in the regulation of rapid eye movement sleep. *J. Biosci.*, 27: 539–551.

- Manfredi, R.L., Kales, A., Vgontzas, A.N., Bixler, E.O., Isaac, M.A. and Falcone, C.M. (1991) Buspirone: sedative or stimulant effect? *Am. J. Psychiatry*, 148: 1213–1217.
- Mansour, A. and Watson, S.J. (1995) Dopamine receptor expression in the central nervous system. In: Bloom F.E. and Kupfer D.J. (Eds.), *Neuropsychopharmacology: The Fourth Generation of Progress*. Raven Press, New York, pp. 207–219.
- Martin, J.R., Börs, M., Jenck, F., Moreau, J., Mutel, V., Sleight, A.J., Wichmann, J., Andrews, J.S., Berendsen, H.H., Broekkamp, C.L., Ruigt, G.S., Köhler, C. and Delft, A.M. (1998) 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J. Pharmacol. Exp. Ther.*, 286: 913–924.
- McCarley, R.W., Greene, R.W., Rainnie, D. and Portas, C.M. (1995) Brainstem modulation and REM sleep. *Sem. Neurosci.*, 7: 341–354.
- McCarley, R.W. and Hobson, J.A. (1975) Neuronal excitability modulation over the sleep cycle: a structural and mathematical model. *Science*, 189: 58–60.
- Meador-Woodruff, J.H. (1995) Neuroanatomy of dopamine receptor gene expression: potential substrates for neuropsychiatric illness. *Clin. Neuropharmacol.*, 18(Suppl. 1): 514–524.
- Mengod, G., Nguyen, H., Le, H., Waeber, C., Lubbert, H. and Palacios, J.M. (1990) The distribution and cellular localization of the serotonin 1C receptor mRNA in the rodent brain examined by in situ hybridization histochemistry. Comparison with receptor binding distribution. *Neuroscience*, 35: 577–591.
- Missale, C., Nash, S.R., Robinson, S.W., Jaber, M. and Caron, M.G. (1998) Dopamine receptors: from structure to function. *Physiol. Rev.*, 78: 189–225.
- Monaca, C., Boutrel, B., Hen, R., Hamon, M. and Adrien, J. (2003) 5-HT_{1A/B} receptor-mediated effects of the selective serotonin reuptake inhibitor citalopram, on sleep: studies in 5-HT_{1A} and 5-HT_{1B} knockout mice. *Neuropharmacology*, 28: 850–856.
- Monti, J.M. (1987) Disturbances of sleep and wakefulness associated with the use of antihypertensive agents. *Life Sci.*, 41: 1979–1988.
- Monti, J.M. (2004) Primary and secondary insomnia: prevalence, causes and current therapeutics. *Curr. Med. Chem: CNS Agents*, 4: 119–137.
- Monti, J.M., Fernández, M. and Jantos, H. (1990a) Sleep during acute dopamine D₁ agonist SKF 38393 or D₁ antagonist SCH 23390 administration in rats. *Neuropsychopharmacology*, 3: 153–162.
- Monti, J.M., Hawkins, M., Jantos, H., D'Angelo, L. and Fernández, M. (1988) Biphasic effects of dopamine D-2 receptor agonists on sleep and wakefulness in the rat. *Psychopharmacology*, 95: 395–400.
- Monti, J.M. and Jantos, H. (1992) Dose-dependent effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on sleep and wakefulness in the rat. *J. Sleep Res.*, 1: 169–175.
- Monti, J.M. and Jantos, H. (2003) Differential effects of the 5-T_{1A} receptor agonist flesinoxan given locally or systemically on REM sleep in the rat. *Eur. J. Pharmacol.*, 478: 121–130.
- Monti, J.M. and Jantos, H. (2004) Effects of the 5-HT_{1A} receptor ligands flesinoxan and WAY 100635 given systemically or microinjected into the laterodorsal tegmental nucleus on REM sleep in the rat. *Behav. Brain Res.*, 151: 159–166.
- Monti, J.M. and Jantos, H. (2005) A study of the brain structures involved in the acute effects of fluoxetine on REM sleep in the rat. *Int. J. Neuropsychopharmacol.*, 8: 75–86.
- Monti, J.M. and Jantos, H. (2006a) Effects of the 5-HT₇ receptor antagonist SB-269970 microinjected into the dorsal raphe nucleus on REM sleep in the rat. *Behav. Brain Res.*, 167: 245–250.
- Monti, J.M. and Jantos, H. (2006b) Effects of the serotonin 5-HT_{2A/2C} receptor-agonist DOI and of the selective 5-HT_{2A} or 5-HT_{2C} receptor antagonists EMD 281014 and SB-243213, respectively, on sleep and waking in the rat. *Eur. J. Pharmacol.*, 553: 163–170.
- Monti, J.M. and Jantos, H. (2006c) Effects of activation and blockade of 5-HT_{2A/2C} receptors in the dorsal raphe nucleus on sleep and waking in the rat. *Progr. Neuro-Psychopharmacol. Biol. Psychiatry*, 30: 1189–1195.
- Monti, J.M., Jantos, H. and Fernández, M. (1989) Effects of the selective dopamine D-2 receptor agonist, quinpirole on sleep and wakefulness in the rat. *Eur. J. Pharmacol.*, 169: 61–66.
- Monti, J.M., Jantos, H. and Monti, D. (2000) Dorsal raphe nucleus administration of 5-HT_{1A} receptor agonist and antagonists: effect on rapid eye movement sleep in the rat. *Sleep Res. Online*, 3: 29–34.
- Monti, J.M., Jantos, H. and Monti, D. (2002) Increased REM sleep after intra-dorsal raphe nucleus injection of flesinoxan or 8-OH-DPAT: prevention with WAY 100635. *Eur. Neuropsychopharmacol.*, 12: 47–55.
- Monti, J.M., Jantos, H., Silveira, R., Reyes-Parada, M. and Scorza, C. (1995a) Sleep and waking in 5,7-DHT-lesioned or (–)pindolol-pretreated rats after administration of buspirone, ipsapirone, or gepirone. *Pharmacol. Biochem. Behav.*, 52: 305–312.
- Monti, J.M., Jantos, H., Silveira, R., Reyes-Parada, M., Scorza, C. and Prunell, G. (1994) Depletion of brain serotonin by 5,7-DHT: effects on the 8-OH-DPAT-induced changes of sleep and waking in the rat. *Psychopharmacology*, 115: 273–277.
- Monti, J.M. and Monti, D. (2000) Histamine H₁ receptor antagonists in the treatment of insomnia: is there a rational basis for use? *CNS Drugs*, 13: 87–96.
- Monti, J.M. and Monti, D. (2004) Sleep in schizophrenia patients and the effects of antipsychotic drugs. *Sleep Med. Rev.*, 8: 133–148.
- Monti, J.M. and Monti, D. (2007) The involvement of dopamine in the modulation of sleep and waking. *Sleep Med. Rev.*, 11: 113–133.
- Monti, J.M., Monti, D., Jantos, H. and Ponzoni, A. (1995b) Effects of selective activation of the 5-HT_{1B} receptor with CP-94,253 on sleep and wakefulness in the rat. *Neuropharmacology*, 34: 1647–1651.
- Monti, J.M., Orellana, C., Boussard, M., Jantos, H., Labraga, P., Olivera, S. and Alvarino, F. (1990b) 5-HT receptor agonists 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

- (DOI) and 8-OH-DPAT increase wakefulness in the rat. *Biogen. Amines*, 7: 145–151.
- Monti, J.M., Ponzoni, A., Jantos, H., Lagos, P., Silveira, R. and Banchero, P. (1999) Effects of accumbens *m*-chlorophenylbiguanide microinjection on sleep and waking in intact and 6-hydroxydopamine-treated rats. *Eur. J. Pharmacol.*, 364: 89–98.
- Moore, R.Y. and Bloom, F.E. (1978) Central catecholamine neuron system: anatomy and physiology of the dopamine systems. In: Cowan W.M., Hall Z.W. and Kandel E.R. (Eds.), *Annual Review of Neuroscience*, Vol. 1. Annual Reviews, Inc., Palo Alto, pp. 129–169.
- Morales, M., Battenberg, E. and Bloom, F.E. (1998) Distribution of neurons expressing immunoreactivity for the 5-HT₃ receptor subtype in the rat brain and spinal cord. *J. Comp. Neurol.*, 402: 385–401.
- Murray, A.M., Ryoo, H.L., Gurevich, E. and Joyce, J.N. (1994) Localization of dopamine D₃ receptors to mesolimbic and D₂ receptors to mesostriatal regions of human forebrain. *Proc. Natl. Acad. Sci. USA*, 91: 1271–1275.
- Neumaier, J.F., Sexton, T.J., Yracheta, J., Diaz, A.M. and Brownfield, M. (2001) Localization of 5-HT₇ receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. *J. Chem. Neuroanat.*, 21: 63–73.
- Newberry, N.R., Footitt, D.R., Papanastassiou, V. and Reynolds, D.J. (1999) Action of 5-HT on human neocortical neurons in vitro. *Brain Res.*, 833: 93–100.
- Nichols, D.E. (2004) Hallucinogens. *Pharmacol. Ther.*, 10: 131–181.
- Oberndorfer, S., Saletu-Zyhlarz, G. and Saletu, B. (2000) Effects of selective serotonin reuptake inhibitors on objective and subjective sleep quality. *Neuropsychobiology*, 42: 69–81.
- Ongini, E., Bonizzoni, E., Ferri, N., Milani, S. and Trampus, M. (1993) Differential effects of dopamine D-1 and D-2 receptor antagonist antipsychotics on sleep-wake patterns in the rat. *J. Pharmacol. Exp. Ther.*, 266: 726–731.
- Ongini, E., Caporali, M.G. and Massotti, M. (1985) Stimulation of dopamine D-1 receptors by SKF 38393 induces EEG desynchronization and behavioral arousal. *Life Sci.*, 37: 2327–2333.
- Overton, P.G. and Clark, D. (1997) Burst firing in midbrain dopaminergic neurons. *Brain Res. Rev.*, 25: 312–334.
- Pace-Schott, E.F. and Hobson, J.A. (2002) Basic mechanisms of sleep: New evidence on the neuroanatomy and neuromodulation of the NREM-REM cycle. In: Charney D. and Nemeroff C. (Eds.), *Neuropsychopharmacology – The Fifth Generation of Progress*. Lippincott Williams and Wilkins, Philadelphia, pp. 1859–1877.
- Parnavelas, J.G. (1990) Neurotransmitters in the cerebral cortex. In: Uylings H.B.M., van Eden C.G., De Bruin J.P.C., Corner M.A. and Feenstra M.G.P. (Eds.), *Progress in Brain Research*, Vol. 85. Elsevier, Amsterdam, pp. 13–29.
- Pehec, E.A., McFarlane, H.G., Maguschak, K., Price, B. and Pluto, C.P. (2001) M100,907, a selective 5-HT_{2A} antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Res.*, 888: 51–59.
- Pompeiano, M., Palacios, J.M. and Mengod, G. (1994) Distribution of the serotonin 5-HT₂ receptor family mRNAs: comparison between 5-HT_{2A} and 5-HT_{2C} receptors. *Molec. Brain Res.*, 23: 163–178.
- Ponzoni, A., Monti, J.M. and Jantos, H. (1993) The effects of selective activation of the 5-HT₃ receptor with *m*-chlorophenylbiguanide on sleep and wakefulness in the rat. *Eur. J. Pharmacol.*, 249: 259–264.
- Ponzoni, A., Monti, J.M., Jantos, H., Altier, H. and Monti, D. (1995) Increased waking after intra-accumbens injection of *m*-chlorophenylbiguanide: prevention with serotonin or dopamine receptor antagonists. *Eur. J. Pharmacol.*, 278: 111–115.
- Portas, C.M., Thakkar, M., Rainnie, D. and McCarley, R.W. (1996) Microdialysis perfusion of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) into the dorsal raphe nucleus decreases serotonin release and increases rapid eye movement sleep in the freely moving rat. *J. Neurosci.*, 16: 2820–2828.
- Portas, C.M., Thakkar, M., Rainnie, D.G., Greene, R.W. and McCarley, R.W. (1997) Role of adenosine in behavioral state modulation: a microdialysis study in the freely moving cat. *Neuroscience*, 79: 225–235.
- Pungor, K., Papp, M., Kékesi, K. and Juhász, G. (1990) A novel effect of MPTP: the selective suppression of paradoxical sleep in cats. *Brain Res.*, 525: 310–314.
- Riad, M., García, S., Watkins, K.C., Jodoin, N., Doucet, E., Langlois, X., El Mestikawy, S., Hamon, M. and Descarries, L. (2000) Somatodendritic localization of 5-HT_{1A} and preterminal axonal localization of 5-HT_{1B} serotonin receptors in adult rat brain. *J. Comp. Neurol.*, 417: 181–194.
- Roberts, C., Thomas, D.R., Bate, S.T. and Kew, J.N.C. (2004) GABAergic modulation of 5-HT₇ receptor-mediated effects on 5-HT efflux in the guinea-pig dorsal raphe nucleus. *Neuropharmacology*, 46: 935–941.
- Rye, D.B. and Jankovic, J. (2002) Emerging views of dopamine in modulating sleep/wake state from an unlikely source: PD. *Neurology*, 58: 341–346.
- Salin-Pascual, R.J., Herrera-Estrella, M., Galicia-Polo, L. and Laurabaquio, M.R. (1999) Olanzapine acute administration in schizophrenic patients increases delta sleep and sleep efficiency. *Biol. Psychiatry*, 46: 141–143.
- Sanford, L.D., Ross, R.J., Seggos, A.E., Morrison, A.R., Ball, B.A. and Mann, G.L. (1994) Central administration of two 5-HT receptor agonists: effect on REM sleep initiation and PGO waves. *Pharmacol. Biochem. Behav.*, 49: 93–100.
- Saper, C.B., Sherin, J.E. and Elmquist, J.K. (1997) Role of the ventrolateral preoptic area in sleep induction. In: Hayaishi O. and Inoué S. (Eds.), *Sleep and Sleep Disorders: From Molecule to Behavior*. Academic Press, Tokyo, pp. 281–294.
- Sari, Y., Lefèvre, K., Bancila, M., Quignon, M., Miquel, M.C., Langlois, X., Hamon, M. and Vergé, D. (1997) Light and electron microscopic immunocytochemical visualization of 5-HT_{1B} receptors in the rat brain. *Brain Res.*, 760: 281–286.
- Sari, Y., Miquel, M.C., Brisorgueil, M.J., Ruiz, G., Doucet, E., Hamon, M. and Vergé, D. (1999) Cellular and subcellular localization of 5-hydroxytryptamine_{1B} receptors in the rat

- central nervous system: immunocytochemical, autoradiographic and lesion studies. *Neuroscience*, 88: 899–915.
- Schäffer, J. and Greulich, A. (2000) Effects of parkinsonian medication on sleep. *J. Neurol.*, 247(Suppl. 4): IV24–IV27.
- Schultz, W. (1998) Predictive reward signal of dopamine neurons. *J. Neurophysiol.*, 80: 1–27.
- Seamans, J.K., Gorelova, N., Durstewitz, D. and Yang, C.R. (2001) Bidirectional dopamine modulation of GABAergic inhibition in prefrontal cortical pyramidal neurons. *J. Neurosci.*, 21: 3628–3638.
- Sebban, C., Zhang, X.Q., Tesolin-Decros, B., Millan, M.J. and Spedding, M. (1999) Changes in EEG spectral power in the prefrontal cortex of conscious rats elicited by drugs interacting with dopaminergic and noradrenergic transmission. *Br. J. Pharmacol.*, 128: 1045–1054.
- Seidel, W.F., Cohen, S.A., Bliwise, N.G. and Dement, W.C. (1985) Buspirone: an anxiolytic without sedative effect. *Psychopharmacology*, 87: 371–373.
- Sharpley, A.L., Elliot, J.M., Attenburrow, M.J. and Cowen, P.J. (1994) Slow wave sleep in humans: role of 5-HT_{2A} and 5-HT_{2C} receptors. *Neuropharmacology*, 33: 467–471.
- Sibley, D.R. and Monsma, F.J. (1992) Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.*, 13: 61–69.
- Silhol, S., Glin, L. and Gottesmann, C. (1991) Study of the 5-HT₂ antagonist ritanserin on sleep-waking cycle in the rat. *Physiol. Behav.*, 41: 241–243.
- Smith, M.I., Piper, D.C., Duxon, M.S. and Upton, N. (2002) Effect of SB-243213, a selective 5-HT_{2C} receptor antagonist, on the rat sleep profile: a comparison to paroxetine. *Pharmacol. Biochem. Behav.*, 71: 599–605.
- Sorensen, E., Gronli, J., Bjorvatn, B., Bjorkum, A. and Ursin, R. (2001) Sleep and waking following microdialysis perfusion of the selective 5-HT_{1A} receptor antagonist *p*-MPPI into the dorsal raphe nucleus of the freely moving rat. *Brain Res.*, 897: 122–130.
- Staner, L., Luthringer, R. and Macher, J.P. (1999) Effects of antidepressant drugs on sleep EEG in patients with major depression. *CNS Drugs*, 11: 49–60.
- Stanford, S.C. (2001) 5-Hydroxytryptamine. In: Webster R.A. (Ed.), *Neurotransmitters, Drugs and Brain Function*. Wiley, Chichester, pp. 187–209.
- Sterman, M.D. and Clemente, C.D. (1962) Forebrain inhibitory mechanisms: sleep patterns induced by basal forebrain stimulation in the behaving cat. *Exp. Neurol.*, 6: 103–117.
- Strecker, R.E., Moriarty, S., Thakkar, M.M., Porkka-Heiskanen, T., Basheer, R., Dauphin, L.J., Rainnie, D.G., Portas, C.M., Greene, R.W. and McCarley, R.W. (2000) Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. *Behav. Brain Res.*, 115: 183–202.
- Szymusiak, R., Steininger, T., Alam, N. and McGinty, D. (2001) Preoptic area sleep-regulating mechanisms. *Arch. Ital. Biol.*, 139: 77–92.
- Takada, M., Li, Z.K. and Hattori, T. (1987) Intracerebral MPTP injections in the rat cause cell loss in the substantia nigra, ventral tegmental area and dorsal raphe. *Neurosci. Lett.*, 78: 145–150.
- Thakkar, M.M., Strecker, R.E. and McCarley, R.W. (1998) Behavioral state control through differential serotonergic inhibition of the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. *J. Neurosci.*, 18: 5490–5497.
- Thomas, D.R. and Hagan, J.J. (2004) 5-HT₇ receptors. *Curr. Drug Targets CNS Neurol. Dis.*, 3: 81–90.
- Thomas, D.R., Melotto, S., Massagrande, M., Gribble, A.D., Jeffrey, P., Stevens, A.J., Deeks, N.J., Eddershaw, P.J., Fenwick, D.N., Riley, G., Stean, T., Scott, C.M., Hill, M.J., Middlemiss, D.N., Hagan, J.J., Price, G.W. and Forbes, I.T. (2003) A novel selective 5-HT₇ receptor antagonist, modulates REM sleep in rats. *Br. J. Pharmacol.*, 139: 705–714.
- Tissier, M.H., Franc, B., Hamon, M. and Adrien, J. (1990) Effects of 5-HT_{1A} and 5-HT₃-receptor ligands on sleep in the rat. In: Horne J. (Ed.), *Sleep 90*. Pontenagel Press, Bochum, pp. 126–128.
- To, Z.P., Bonhaus, D.W., Eglen, R.M. and Jakeman, L.B. (1995) Characterization and distribution of putative 5-HT₇ receptors in guinea-pig brain. *Br. J. Pharmacol.*, 115: 107–116.
- Tortella, F.C., Echevarria, E., Pastel, R.H., Cox, B. and Blackburn, T.P. (1989) Suppressant effects of selective 5-HT₂ antagonists on rapid eye movement sleep in rats. *Brain Res.*, 485: 294–300.
- Touyz, S.W., Saayman, G.S. and Zabow, T. (1978) A psychophysiological investigation of the long-term effects of clozapine upon sleep patterns of normal young adults. *Psychopharmacology*, 56: 69–73.
- Trampus, M., Ferri, N., Adami, M. and Ongini, E. (1993) The dopamine D₁ agonists A68930 and SKF 38393, induce arousal and suppress REM sleep in the rat. *Eur. J. Pharmacol.*, 235: 83–87.
- Trampus, M. and Ongini, E. (1990) The D₁ dopamine receptor antagonist SCH 23390 enhances REM sleep in the rat. *Neuropharmacology*, 29: 889–893.
- Trulson, M.E. and Jacobs, B.L. (1979) Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Res.*, 163: 135–150.
- Vertes, R.P. and Kocsis, B. (1994) Projections of the dorsal raphe nucleus to the brainstem: PHA-L analysis of the rat. *J. Comp. Neurol.*, 340: 11–26.
- Vertes, R.P., Fortin, W.J. and Crane, A.M. (1999) Projections of the median raphe nucleus in the rat. *J. Comp. Neurol.*, 407: 555–582.
- Wang, R.Y. and Aghajanian, G.K. (1977) Antidromically identified serotonergic neurons in the rat midbrain raphe: evidence for collateral inhibition. *Brain Res.*, 132: 186–193.
- Weiner, D.M., Levey, A.I., Sunahara, R.K., Niznik, H.B., O'Dowd, B.F., Seeman, P. and Brann, M.R. (1991) D₁ and D₂ dopamine receptor mRNA in rat brain. *Proc. Natl. Acad. Sci. USA*, 88: 1859–1863.
- White, F.J. (1996) Synaptic modulation of mesocorticolimbic dopamine neurons. *Annu. Rev. Neurosci.*, 19: 405–436.

- Wisor, J.P., Nishino, S., Sora, I., Uhl, G.H., Mignot, E. and Edgar, D.M. (2001) Dopaminergic role in stimulant-induced wakefulness. *J. Neurosci.*, 21: 1787–1794.
- Yakel, J.L. (2000) The 5-HT₃ receptor channel: function, activation and regulation. In: Endo M. (Ed.), *Pharmacology of Ionic Channel Function: Activators and Inhibitors*. Springer, Berlin, pp. 541–560.
- Yamashita, H., Morinobu, S., Yamakawi, S. and Horiguchi, J. (2002) Effect of risperidone on sleep in schizophrenia: a comparison with haloperidol. *Psychiatry Res.*, 109: 137–142.
- Zoltoski, R.K., Cabeza, R.J. and Gillin, J.C. (1999) Biochemical pharmacology of sleep. In: Chokroverty S. and Daroff R.B. (Eds.), *Sleep Disorders Medicine: Basic Sciences, Technical Considerations and Clinical Aspects* (2nd edn.). Butterworth-Heinemann, Boston, pp. 63–94.

Subject Index

- AADC, *see* amino acid aromatic decarboxylase (AADC)
- AAPA, *see* active allothetic place avoidance task (AAPA)
- abnormal involuntary movements rating scale (AIMS), 481
- accumbens nucleus (AN), 568
- acetylcholine (ACh), 123, 569
- ACh, *see* acetylcholine (ACh)
- ACTH, *see* adrenocorticotrophin (ACTH)
- active allothetic place avoidance task (AAPA), 575
- acute Trp depletion (ATD), 569
- AD, *see* Alzheimer's disease (AD)
- adenosine, 626
- adenylate cyclase, 574
- ADHD, *see* attention-deficit hyperactivity disorder (ADHD)
- adrenocorticotrophin (ACTH), 80
- afferent connections, 627
 - of dorsal raphe nucleus, 629
 - of median raphe nucleus, 629
- afterhyperpolarizations (AHP), 433
- aggression, 308
 - dopamine in, 308–309
 - serotonin in, 308–309
- agomelatine, antidepressant efficacy, 26
- 'agonist' therapy, 389–392
- agranulocytosis, 119–120
- AIMS, *see* abnormal involuntary movements rating scale (AIMS)
- Alzheimer's disease (AD), 5, 250, 581
 - amyloid plaques and neurofibrillary tangles, in hippocampus, 252
 - BDNF mRNA and protein levels, 251
 - cortisol levels in, 252
 - depression and, 252–254
 - DRN neuron loss in, 253
 - hippocampal damage in, 252
 - hippocampal neurogenesis, 253
 - neuroplasticity in, 251–252
 - related depression and cerebral blood flow, 253
 - serotonergic transmission in, 252
- amino acid, 569
- amino acid aromatic decarboxylase (AADC), 466
- amisulpride, antipsychotic drugs, 120, 129, 216
- amitriptyline, 25
- AMPA receptor, 614
- amperozide, 16, 125, 126
- amphetamine, 145, 146, 147, 148, 386, 544, 554
 - induced DA release in striatum, 219
 - and paranoid psychosis, 213
- AN, *see* accumbens nucleus (AN)
- anatomic localization, of TS, 496, *see also* tourette syndrome (TS)
- anhedonia, 268
- animal models
 - mesolimbic dopamine in, 266–268
 - of psychostimulant abuse, 326, 327
- antidepressant-induced behavioural changes, 247
- antidepressants
 - chronic administration of, 267–268, 269–270
 - desipramine, *see* desipramine
 - and D2-like receptor antagonists, 266–267
 - methylphenidate, 266
 - and neuronal firing activity, *see* cell firing
 - nomifensine, 266
 - onset time of, 270–272
 - paroxetine, 268–269
 - and VTA, 274–275
- antipsychotic drugs (APD), 13, 424, 638
 - 5-HT_{2A} and DA D₂ receptor antagonism, 178–185
 - partial DA agonist, 184–185
 - for Schizophrenia, 27–28
 - in vivo actions and 5HT_{2A} receptors, 185–186

- antipsychotic medications, 141, 144, 412
 - antipsychotic drugs (APD), 13
 - 5-HT_{2A} and DA D₂ receptor antagonism, 178–185
 - partial DA agonist, 184–185
 - for Schizophrenia, 27–28
 - in vivo actions and 5-HT_{2A} receptors, 185–186
 - antipsychotic efficacy
 - atypical antipsychotic medications, 171–172
 - data analysis, 157
 - methods of, 157
 - roles of serotonin and dopamine receptor, in binding, 155
 - typical antipsychotic medications, 168–171
 - atypical, 171
 - dosage and binding affinity ratio, 168–169
 - limitations, 171–172
 - typical, 168–171
- antisocial behaviour (ASB), 85
- anti-stimulant effects of 5-HT_{2C} receptor
 - activation, 392
- anxiety, and 5-HT_{2C} receptors, 288–289
- anxiety-related behavioural changes induced by
 - serotonergic activity, 236
- APD, *see* atypical antipsychotic drugs (APD)
- apomorphine, 125, 127, 632
- aripiprazole antipsychotic drugs, 16, 120, 122, 127, 129, 216–217
 - DA agonist, 179, 184–187
 - partial D₂ agonist, dual effects on dopamine release in medial prefrontal cortex, 207
- ASB, *see* antisocial behaviour (ASB)
- astroglia/microglia culture, 557
- ATD, *see* acute Trp depletion (ATD)
- attentional deficit indexing, 520–521, *see also* attentional processes
- attentional processes
 - attentional deficit indexing, 520–521
 - 5CSRTT, 521–526
 - defined, 517–518
 - neuropsychiatric disorders, in, 518–519
 - PFC in anatomical substrate, 519–520
 - set-shifting, 526–532
 - SSRT, 532–534
- attention-deficit hyperactivity disorder (ADHD),
 - 5, 75, 496, 518, 519, 543
 - of clinical problems, 543–544
 - vs.* conduct disorder, 557
 - putative dopamine and serotonin interactions, 548–549
- atypical antipsychotic drugs (APD), 587
- atypical antipsychotic medications
 - atypical antipsychotics, 119–120
 - limbic selectivity, 120
 - D₃ subtype DA receptors and, 171
 - 5-HT_{2A} and 5-HT_{2C} receptors, 171
- autoreceptors, 349–350
 - cocaine-induced behaviour and, 352–353, 354
 - and locomotor activity, 350–351
- axospine synapses, 612
- BA, *see* Brodmann's area (BA)
- bacterial leucine transporter, 221
- basal dopamine, 124
- basal extracellular levels, of dopamine, 268
- basal ganglia, 579
 - and 5-HT, in PD, 481–490
 - 5-HT innervation, 424–426
 - 5-HT receptor distribution, 426–431
- BDNF, *see* brain-derived neurotrophic factor (BDNF)
- benzodiazepine, 142
- bicuculline, 415, 586
- Biel's maze, 570
- Bifuprenox, 122, 130
- BIMG 80, 16
- blood oxygen level-dependent (BOLD), 82, 527
- BOLD, *see* blood oxygen level-dependent (BOLD)
- brain
 - autoreceptors, 349–350
 - dopamine in, 348–349
 - regions, roles of, 626–627
 - role of DRN as modulator, 236
 - serotonin in, 348–349
- brain-derived neurotrophic factor (BDNF), 83, 246
 - antidepressant therapy and neurogenesis, 247
 - depression disorders and, 82
 - exercise and, 251
 - gene expression and dietary restriction, 251
 - mRNA expression, 185
 - and receptor trkB, in ageing brain, 251

- Brodmann's area (BA), 101, 584
- bromocriptine, 632
- dopaminergic receptor agonists, 221
- bupropion, 362
- DA and noradrenaline re-uptake inhibitor, 221
- burst, *see also* cell firing, in VTA
- defined, 275
- firing, 121, 273, 275–277
- influence, 277–278
- buspirone, 5-HT_{1A} receptor partial agonist drugs, 217
- calbindin (CB) cells, 103, 107
- immunostaining, 427
- cAMP responsive element (CREB) of
- cAMP-signalling cascade, 246
- cannabinoids, 417
- CAPSIT-PD, *see* Core Assessment Program for
- Surgical Interventional Therapies in PD (CAPSIT-PD)
- CAR, *see* Conditioned avoidance response (CAR),
- animal model
- 5-carboxamindotryptamine (5-CT), 440
- castellanos CSF studies, 552
- CAT, *see* Chloramphenicol acetyltransferase (CAT)
- catecholamine, 544
- receptor, 167
- catechol-*O*-methyltransferase, (COMT) gene, 80–82
- val158Met polymorphism, 80–82
- CB, *see* Calbindin (CB) cells
- CCK, *see* Cholecystokinin (CCK)
- CeA, *see* Central nucleus of amygdala (CeA)
- cell firing, in VTA, *see also* interspike interval (ISI), in VTA
- and antidepressants, 274–275
- and burst influence, 277–278
- firing patterns, 275–277
- modes of, 273–274
- central dopamine (DA) systems, 3
- central nervous system (CNS), 423, 567, 611
- central nucleus of amygdala (CeA), 236
- centre-surrounded model, of basal ganglia function, 499, *see also* tourette syndrome (TS)
- cerebral 5-HT manipulation, 568–572
- cerebral serotonergic depletion, 569
- cerebrospinal fluid (CSF), 504
- CG1, *see* cingulate anterior (CG1)
- ChIP, *see* chromatin immune precipitation (ChIP)
- chloramphenicol acetyltransferase (CAT), 75
- chlorpromazine (CPZ), antipsychotic drugs, 118, 119, 129, 158–163, 165–169, 216
- APDs and, 27, 178–179, 182, 187
- and 5-HT_{2A} receptor blockade, 218
- cholecystokinin (CCK)
- containing neurons, 244
- immunoreactivity, 12
- cholinergic cells, 627
- cholinergic neurons, 626
- cholinergic system, 608
- chromatin immune precipitation (ChIP), 80
- cingulate anterior (CG1), 584
- citalopram, 555, 634
- Citalopram drug, 123
- chronic treatment for, 26
- clinical implications, of 5-HT/DA interaction, 588–591
- clinical studies
- 5-HT_{1A} agonists, 486–487
- SSRI, 490
- clock-like neurons, 235
- clozapine, 589
- clozapine drug, 16, 19, 104, 109, 110, 119, 122, 124, 125, 146, 180–186, 188–189
- anticholinergic activity of, 128
- cognitive symptoms and, 129
- D2 antagonism and, 120
- extrapyramidal symptoms (EPS) and, 128–129, 215
- negative symptoms and, 129
- neuroleptic drug, 83
- positive symptoms and, 128
- refractory schizophrenia, treatment of, 128
- related antipsychotic drugs, 178–179
- serotonin–dopamine antagonism and, 119
- transient occupancy of brain DA D₂ receptors, 216
- CMV, *see* cytomegalovirus (CMV) promoter
- CNS, *see* central nervous system (CNS)
- cocaine, 146, 554
- cocaine psychosis, 213
- 5-HT_{1B} receptor agonists, 30
- and 5HT receptors, 332–333
- cocaine-induced behaviour, 352–354
- locomotor activity in, 352–353

- COGA, *see* collaborative study on genetics of alcoholism (COGA)
- collaborative study on genetics of alcoholism (COGA), 87
- COMT, *see* Catechol-*O*-methyltransferase, (COMT) gene
- conditioned avoidance response (CAR), 125
animal model, 125–127
- conditioned place preference (CPP)
assay, 390
and 5HT_{2A} receptor, 330–332
and 5HT_{2C} receptor, 330–332
paradigm, 412–413
- conduct disorder *vs.* ADHD, 557
- Conners scale, 545
- constitutive receptor activity, 291–292
5-HT_{2A} receptors
in vitro, 292–293
in vivo, 294–296
5-HT_{2C} receptors
in vitro, 292–294
in vivo, 296–298
- continuous performance task (CPT), 552
- Core Assessment Program for Surgical Interventional Therapies in PD (CAPSIT-PD), 481
- corticotropin-releasing factor (CRF), 236
and HPA axis in depression, 250
immunoreactive neurons, 244
- CP 93129, for DA release, 17
- CP-94253, 467
- CPT, *see* continuous performance task (CPT)
- CPZ, *see* chlorpromazine (CPZ)
- CRF, *see* corticotropin-releasing factor (CRF)
- 5CSRTT, *see* five-choice serial reaction time task (5CSRTT)
- 5-CT, *see* 5-carboxamindotryptamine (5-CT)
- cytokines, 557
- cytomegalovirus (CMV) promoter, 78
- DA, *see* dopamine (DA)
- DArgic cells, 585
- DArgic system, 572
- DAT, *see* dopamine transporter (DAT)
- DDC, *see* dopa decarboxylase (DDC)
- deep brain stimulation (DBS), 500
- dementia, 579
- dendrites, 613
- dendritic calcium spikes, 60
- dendritic spines, in learning and memory, 613–616
- dentate gyrus, 610
- depression
dopamine in, 309–310
5-HT_{2A} receptors, 289–290
5-HT_{2C} receptors, 288–289
serotonin in, 309–310
- depression, dopamine in, *see also* antidepressants
basal extracellular levels of, 268
bioavailability of, 270–272
mesolimbic DA, *see* mesolimbic dopamine, in depression
and NAc, 268–270
serotonin, 268–270
VTA, *see* ventral tegmental area, in depression
- depression, neuropsychiatric disorders, 8–9, 24
Alzheimer's disease (AD), 252–254
antianhedonic effects and, 25
antidepressants for 5-HT_{2C} receptors, 25
BDNF and, 82
C281A polymorphism and, 247
DRN and, 246–254
5-HT_{1A} receptors promoter (C1019G)
polymorphism, 248
5-HT_{2C} agonists, role, 25
monoamine hypothesis, 219
neuroplasticity and, 246–248
rauwolfia alkaloid reserpine and, 213
serotonin-dopamine interactions and, 24
serotonin (5-HT) and, 246
tryptophan hydroxylase 2 and, 249
- depressive behavior, *see* depression
- desipramine, 268
chronic treatment with, 269
and mesolimbic neuronal activity, 272–276, 279
vs. nefazodone, 271
- desipramine drug, 221
- 5,7-dihydroxytryptamine (5,7-DHT), 466
- 1-[2,5-Dimethoxy-4-iodophenyl]-2-aminopropane] (DOI), 124
- diskynesias, 5
- DLPFC, *see* dorsolateral prefrontal cortex (DLPFC)
- DOI, *see* 1-[2,5-Dimethoxy-4-iodophenyl]-2-aminopropane] (DOI)
- DOM, *see* 4-methyl-2,5-dimethoxyamphetamine (DOM)
- dopa decarboxylase (DDC), 550

- dopamine (DA), 543, 545, 568, 605
- in aggression, 308–309
 - in behavioural state modulation, 630–633
 - DA receptor agonists and antagonists, 631–633
 - DA transporter, 630–631
 - neurotoxin-provoked cell, 631
 - in brain, 348–349
 - autoreceptors, 349–350
 - and cocaine-induced behaviour, 352–354
 - DA D₂ receptors
 - antagonism, 218
 - aripiprazole, 217
 - in brain, 216
 - DA D₂-family receptor-binding, 156
 - D₃ receptor blockade in striatum, 216
 - occupancy in cortex with antipsychotic drug treatment, 216
 - occupancy in striatum, 218
 - D₂ agonists, 146
 - in depression, 309–310
 - D₂ (5-HT_{2A}/5-HT_{1A}) receptor-binding affinities, 155, 164, 165, 167, 171
 - D₃ (5-HT_{2A}/5-HT_{1A}) receptor-binding affinities., 155, 164, 165
 - D₂/5-HT_{1A} receptor binding affinities, 155, 162
 - D₃/5-HT_{1A} receptor binding affinities, 155, 162
 - D₂ (5-HT_{2C}/5-HT_{1A}) receptor-binding affinities, 155, 164, 165, 169, 171
 - D1-like receptors
 - cellular localization in PFC and, 108
 - D1 and D5 mRNAs, 108
 - mRNAs coding for, 109
 - D2-like receptors
 - cellular localization in PFC and, 108–109
 - D2 and D4 mRNA, 109
 - dopamine/5-HT_{2A} and 5-HT_{2C} receptor interactions, 217–218
 - dopamine/5-HT_{1A} receptor interaction, 217
 - dopamine/5-HT₆ receptor interactions, 218–219
 - dopaminergic neurons pathways, 120
 - and 5-HT
 - DA-ergic lesion on, neuron activity, 49
 - inhibition of, 49
 - interaction, 54
 - hyperdopaminergic activity in, 121
 - hypersensitivity and, 121
 - hypothesis, of TS, 500–501
 - tonic-phasic, 503–504
 - in investigations of ADHD, 549–554
 - genetics, 549–551
 - neurochemistry, 551–552
 - neuropsychology, 552–554
 - laminar and cellular localization of, 104
 - in learning and memory, 611–613
 - MDMA -induced, release, 18
 - mesolimbic DA pathway, 8
 - neurobiology, 120–122
 - neuronal activity
 - DRN and MRN, electrical stimulation of, 53–54
 - serotonergic control of, 54–59
 - serotonergic lesion on, 50–51
 - serotonin reuptake inhibitors, 51–52
 - neuronal function, 144
 - neuron firing rate, 18
 - neurotransmission of, 8, 144
 - receptor agonists and antagonists, 631–633
 - receptors, 121, 123, 157, 627–628
 - classification of, 107–108
 - D₂, 144, 148, 155, 216–217
 - D₃, 141, 145, 146, 155
 - D₃, antagonists, 141, 143
 - D₃, DRD3, 74
 - D₃, mRNA, 146
 - D₃, protein, 145
 - D₄, DRD4, 74, 75, 77
 - DA D₃, 156
 - DA D₄, 156
 - D₁ and D₂ family receptors, 145
 - receptors and cognition, 574–575
 - serotonergic neurons activity, modulation, 48
 - in sleep and waking regulation, 638–639
 - and spontaneous behaviour, 350–352
 - striatal DA pathways, 8
 - in suicidal behaviour, 309–310
 - symptoms and side effects, 118
 - and TS treatment, 501–502
 - ventral mesencephalon neurons of, 8
 - in vitro electrophysiological studies, 60–62
 - dopamine (DA), in depression, *see also* antidepressants
 - basal extracellular levels of, 268
 - bioavailability of, 270–272

- mesolimbic, *see* mesolimbic dopamine, in
 - depression
 - and NAc, 268–270
 - serotonin, 268–270
 - VTA, *see* ventral tegmental area, in depression
- dopaminergic (DAergic) systems, 568
- dopaminergic fibres, 613
- dopaminergic manipulation effects
 - 5CSRTT, 523
 - set-shifting, 528–530
 - SSRT, 533
- dopaminergic nerve terminals, 605
- dopaminergic neurotransmission
 - functional imaging, 502–503
 - and 5HT receptors, 325–326
 - of psychostimulant-evoked, 326
 - regulation of, 288, 290–291
- dopaminergic nuclei, 627
- dopaminergic system and cognition, 572–574
- dopamine-serotonin interactions, in TS, 506–507,
 - see also* tourette syndrome (TS)
- dopamine transporter (DAT), 127, 497, 545,
 - 630–631
 - DAT1, 77–79
 - post-mortem studies, 78
 - 3'UTR VNTR and, 77–79
 - VNTR polymorphism in, 78
 - DAT blockade into SNRI-like profile, 221
 - SLC6A3, 77–79
- dorsal ascending pathway from medial and rostral
 - DRN
 - striatum and globus pallidus, 238
- dorsal raphe (DR), 9, 431
- dorsal raphe nucleus (DRN), 3, 46, 424, 479,
 - 568
 - ascending pathways, 237
 - cortical tract to substantia nigra (SN) and caudateputamen (CP), 235
 - DA-ergic innervation of, 48
 - depression and Alzheimer's disease, 246–254
 - descending pathways, 237–238
 - efferent projections of, 235–236
 - electrical stimulation on DA neuron activity, 53
 - fibre morphology, 236–237
 - 5-HT neurons, 49
 - medial ascending pathway, 238–239
 - morphology of, 233–234
 - neurons
 - and AD, 251
 - B1–B9 subdivisions, 234
 - Classes I-A and I-B*, 235
 - distribution of, 235
 - types of, 234–235
 - neurotransmitters
 - GABAergic neurons, 243
 - nitric oxide (NO) in, 244
 - peptide transmitters, 244
 - serotonin and dopamine, 243
 - plasticity
 - neurogenesis, 245–246
 - sprouting and synaptogenesis, 245
 - ventral ascending pathway, 239–243
- dorsolateral prefrontal cortex (DLPFC), 101, 103, 110
- DR, *see* dorsal raphe (DR)
- D₂ receptor agonist, 573
- D₁ receptors, 574
- DRN, *see* dorsal raphe nucleus (DRN)
- drug discrimination
 - and 5-HT_{2A} receptor, 329–330
 - and 5-HT_{2C} receptor, 329–330
- drugs
 - abuse, neuropsychiatric disorders, 8–9
 - DA neuron activity, 30
 - 5-HT₃ receptors, role, 30
 - addiction, 5-HT role, 61
- DSM IV, 544
- dual deficit model of stimulant addiction,
 - 387–389
- dyskinesia, 590
 - evaluation, 480–481
 - L-DOPA-induced, 480
 - human, observations on, 471–473
 - MPTP-lesioned monkeys, observations on, 470–471
 - post-synaptic alterations, 473
 - pre-synaptic model, 468–470
 - rats, observations on, 466–468
- ECT, *see* electroconvulsive therapy (ECT) as antidepressant therapy
- EDS, *see* excessive daytime sleepiness (EDS)
- efferent connections, 627
 - of dorsal raphe nucleus, 629
 - of median raphe nucleus, 629

- electroconvulsive therapy (ECT) as antidepressant therapy, 247
 electroencephalogram, 638
 electrophoretic mobility shift assay (EMSA), 78
 electrophysiological studies, 572
 EMSA, *see* electrophoretic mobility shift assay (EMSA)
 ENK, *see* enkephalin (ENK)-containing neurons
 enkephalin (ENK)-containing neurons, 244
 enzyme tryptophan hydroxylase (TPH2), 547
 EPS, *see* extrapyramidal symptoms (EPS)
 EPSC, *see* excitatory postsynaptic currents (EPSC)
 ergotamine, 394
 ERK, *see* extracellular-regulated kinase (ERK)
 escitalopram and SERT occupancies, 221
 ethanol, 5-HT_{1B} receptor agonists, 30
 excessive daytime sleepiness (EDS), 637
 excitatory postsynaptic currents (EPSC), 61, 437, 586
 extracellular-regulated kinase (ERK), 185
 extrapyramidal side effects, 446–449
 extrapyramidal symptoms (EPS), 119, 178
 catalepsy and, 126

 ‘Fast-off’ theory, 203–204
 fenfluramine, 388–389, 392–394, 397
 neurotoxins, 220
 FGAs, *see* first-generation antipsychotic drugs (FGAs)
 fimbria fornix, 569
 firing pattern
 serotonergic neurons, 629
 SNc neurons, 628–629
 VTA neurons, 628–629
 first-generation antipsychotic drugs (FGAs), 200
 five-choice serial reaction time task (5CSRTT), 521–526
 clinical implications, 526
 fronto-striatal lesion effects, 522–523
 neurochemical modulation, 523–526
 DA–5-HT interaction, 525–526
 dopaminergic manipulation effects, 523
 serotonergic manipulation effects, 523–525
 flibanserin, antidepressant agents, 26
 flinders sensitive line (FSL) rats, depression in, 267
 basal extracellular levels of DA, 268
 cell firing, *see* cell firing, in VTA
 mesolimbic neuronal activity, 272–273
 NAc of, 268–270
 fluorodopa, 550
 fluoxetine
 in depression, 266, 268–269
 tricyclic antidepressants, 22, 25, 27, 51
 fluvoxamine, 51–52
 Food and Drug Administration (FDA), 156
 fronto-striatal lesion effects
 5CSRTT, 522–523
 set-shifting, 526–528
 SSRT, 532–533
 functional imaging, of dopaminergic neurotransmission, 502–503

 GABA, *see* γ -aminobutyric acid (GABA)
 GABA_A receptor agonist, 637
 GABAergic cells, *see* gamma-aminobutyric acidergic (GABAergic) cells
 GABAergic interneurons, 609
 GABAergic neurons, 585, 586
 GABA interneurons, 549
 GABA-T, *see* GABA-transaminase (GABA-T)
 GABA-transaminase (GABA-T), 243
 GAD, *see* glutamic acid decarboxylase (GAD)
 GAD67, *see* glutamic acid decarboxylase 67 (GAD67)
 galanin, 577
 colocalization with serotonin in DRN, 244
 γ -Aminobutyric acid decarboxylase, GABA-synthesizing enzyme, 243
 γ -Aminobutyric acid (GABA), 123, 141, 143, 145, 583, 626
 GABA-ergic neuron, 4, 48
 MDMA treatment, release, 18
 gamma-aminobutyric acidergic (GABAergic) cells, 424
 G α_q , 417
 Gaussian-scaled surrogate, 51
 genes
 genBank accession numbers and polymorphic marker locations, 76
 MAOB, 84
 OMIM accession numbers and associated disorders, 75
 genetics, 549–551
 GFP, *see* green fluorescent protein (GFP)
 G α inhibitory proteins, 145

- Gi/o, G protein, 105
- glial cells, 555
- globus pallidus (GP), 424
 - in 5-HT receptor distribution, 429–431
- globus pallidus internus (GPi), 29
- globus pallidus pars interna (GPi), 497
- glucocorticoids, excitotoxicity-induced tangle
 - formation in hippocampus, 253
- glutamate neurotransmission, 482
- glutamatergic cells, 105–107
- glutamic acid decarboxylase (GAD), 47–48
- glutamic acid decarboxylase 67 (GAD67), 103
- glycolysis, 555
- GP, *see* globus pallidus (GP)
- GP activity, in 5-HT modulation, 439–440
- GPi, *see* globus pallidus internus (GPi)
- G-protein coupled receptors (GPCRs), 143, 408, 629
 - G-protein-coupled inwardly rectifying potassium channels (GIRKs), 60–61
- G-protein mediated signal transduction
 - (5-HT_{1A/1B/1D/1E/1F} 5-HT₄, 5-HT₆ and 5-HT₇), 122
- G129R, 410–411
- GR125487, 5-HT₄ antagonist, 59
- grafts, serotonin-rich, 482
- granisetron, 578
- green fluorescent protein (GFP), 78
- Gs stimulatory proteins, 145
- haloperidol, 632, 633
 - antipsychotic drugs, 27, 178–181, 183, 185–189, 202, 216
- herpes simplex virus thymidine kinase (HSV-TK), 78
- hippocampal 5-HT depletion, 569
- histamine in DRN, 245
- histaminergic cell, 629
- HSV-TK, *see* herpes simplex virus thymidine kinase (HSV-TK)
- 5-HT, *see also* serotonin (5-HT)
 - basal ganglia in PD, and, 481–490
 - DA, and, in TS, 500–501
 - innervation, of basal ganglia, 424–426
 - in learning and memory, 607–611
 - in LID, 444–445
 - modulation, of basal ganglia circuitry, 431–440
 - GP activity, 439–440
 - SNr activity, 434–436
 - STN activity, 436–439
 - striatal activity, 431–434
 - OCD treatment, and, 504–505
 - in Parkinsonian resting tremor, 445
 - in Parkinson's disease, 440–441
 - in psychiatric complications, 445–446
 - receptor distribution, within basal ganglia nuclei
 - in striatum, 426–429
 - in substantia nigra and globus pallidus, 429–431
 - in subthalamic nucleus, 431
 - receptors' expression, in animal models, 442–444
 - TS treatment, and, 505–506
- 5-HT_{1A}
 - agonists, 486–487
 - ligands, 482–486
- 5-HT_{2A} antagonists, 488–489
- 5-HT_{2A/2B/2C} receptors, 635–636
 - 5-HT₂ receptor agonists, 635–636
 - 5-HT₂ receptor antagonists, 636
- 5-HT_{2A}/D₂ hypothesis, 178–179, 178–180, 182–183
- 5-HT_{2A}/D₂ receptors, binding potencies of anti-psychotic drugs, 201
- 5-HT₂ antagonists
 - ketanserin, 125
- 5-HT₆ antagonist SB-399885, haloperidol-induced and risperidone-induced dopamine release, 208
- 5-HT_{1A} receptors, 9–10, 148, 482–487, 608, 634–635
 - antagonist WAY100,635, 205
- APDs action, role in, 187
- clozapine and aripiprazole, 205–206
- DA function, modulation of, 55
- and dopamine function, 13–16
- and D₂ receptors, *in vivo* occupancy by antipsychotic drugs, 202–203
- and effects of nicotine, 368–369
- full agonists autoreceptors, 634
- 5-HT_{1A} agonists, clinical studies, 486–487
- and 5-HT_{2A} interactions, 186–187
- MKC-242, receptor agonist, 14
- mRNAs coding for, 106
- 8-OH-DPAT, agonist, 186
- partial agonists at postsynaptic receptors, 634
- PFC, cellular localization, 105–106
- postsynaptic receptors, 634

- and prefrontal dopamine, 204–206
- presynaptic receptors, 634
- promoter (C1019G) polymorphism and depression, 248
- pyramidal neurons, hyperpolarization of, 186
- in raphe nuclei, 214
- serotonin reuptake inhibitors, 634–635
- tandospirone drugs, 206, 217
- 5-HT_{2A} receptors, 4–5, 10, 104–107, 156, 160, 171, 287–288, 488–489
- anatomy and function of, 179
- antagonists, 141
- antipsychotic drug action, role, 183
- APDs efficacy and side effects, 186–189
- clinical trials testing, 182–183
- constitutive receptor activity of
 - in vitro, 292–293
 - in vivo, 294–296
- in cortical pyramidal neurons, 179
- CPP, 330–332
- DA-ergic areas of brain, 47
- DA neurotransmission, 325–326
- D₂ antagonists, prolactin secretion, 183
- and depression, 289–290
- distribution and functions of, 144
- distribution of, 322–323
- DOI receptor agonist, 17
- and dopamine function, 17–20
- and dopaminergic neurotransmission, 290–291
- D2 receptor blockade, 180
- and drug discrimination, 329–330
- and effects of nicotine, 370
- enhanced glutamate efflux, 18
- functional regulation of, 323–325
- His452Tyr allele, 183
- immunoreactivity on non-DA cells, 11
- ligands, 333–336
- and limbic and cortical DA efflux, 179–180
- and locomotor hyperactivity, 326–329
- M100907 receptor agonist, 18
- PFC, cellular localization, 106–107
- in polysynaptic neural circuit, 18
- postmortem and PET studies of, density, 181–182
- preclinical and clinical studies, drugs, 183–184
- pre-psychotic cognitive processes, involvement in, 144
- psychosis, antagonism and animal models of, 180–181
- psychostimulant abuse, *see* psychostimulant abuse
- pyramidal cells and, 107
- in schizophrenia, 182
- self administration, 330–332
- serotonin and dopamine related APDs action, 185–186
- somatodendritic localization of, 11
- SR46349B receptor agonist, 18
- VTA DA neurons, functional role for, 56
- 5-HT_{1B} receptors, 10, 487, 609, 635
- application in mPFC, 17
- cocaine-evoked DA overflow, 17
- and dopamine function, 16
- GR 127935, selective antagonist, 17
- mesocortical pathway regulation, 17
- SB 216641, selective antagonist, 17
- TFMPP, receptor agonists, 55
- 5-HT_{2C}
 - antagonists, 489–490
 - receptors, 489–490
- 5-HT_{2C} receptors, 287–288, 583
- activation, anti-stimulant effects of, 392
- adenosine-to-inosine editing, 5
- affinity, 155
- agonists, 123
- agonist WAY-163909, 218
- antidepressant activity, 24–25
- and anxiety, 288–289
- APDs action, role in, 187–188
- in basal ganglia and dopaminergic system, 215
- behavioural profile of, agonist, 188
- co-expression with GAD, 47–48
- constitutive activity, 48
- constitutive receptor activity of
 - in vitro, 292–294
 - in vivo, 296–298
- CPP, 330–332
- on DA and GABA-containing neurons, 48
- DA neurotransmission, 325–326
 - of psychostimulant-evoked, 326
- and depression, 288–289
- distribution of, 322–323
- and dopamine function, 20
- and dopaminergic neurotransmission, 290–291
- and drug discrimination, 329–330

- and effects of nicotine, 370–373
- family and distribution, 11
- functional regulation of, 323–325
- G α 0/11 χ ουπλινγ, 48
- gene in schizophrenic patients, 208
- 5-HT_{2C}/D₂ binding affinity, 155, 164, 166, 167, 169
- hyposensitivity of, 52
- ligands, 333–336
- and locomotor hyperactivity, 326–329
- motor disorders, therapy of, 58
- and mRNA editing, 289
- mRNAs receptor proteins on neuronal components, 47
- post transcriptional editing, 5
- psychostimulant abuse, *see* psychostimulant abuse
- receptor agonists, 57
- RNA editing, 187
- in schizophrenia, 27–28
- self-administration, 330–332
- subtypes, 11
- third intracellular loop (3L4F), 4
- 5-HT/DA interaction
 - clinical implications of, 588–591
 - in learning and memory, 616–617
 - prefrontal cortex, 586–588
- 5-HT–dopamine antagonists (SDAs), 120
- 5-HT_{1D} receptors, 10
- 5-HT_{1E} receptor subtype, 10–11
- 5-HTergic cells, 571
- HTR2A, *see* 5-Hydroxytryptamine receptor 2A (HTR2A)
- 5-HT₃ receptor, 636–637
- 5-HT₆ receptor, 583
- 5-HT₇ receptor, 637
- 5-HT₂ receptor agonists, 635–636
- 5-HT receptors
 - and cocaine, 332–333
 - 5HT_{2A} receptor, *see* 5-HT_{2A} receptors
 - 5HT_{2C} receptor, *see* 5HT_{2C} receptor
 - psychostimulant abuse, *see* psychostimulant abuse
- 5-HT₁ receptors, 581
- 5-HT₄ receptors
 - and effects of nicotine, 374–375
 - expression, 12
- 5-HT₂ receptors, 144, 581
 - and altanserin, 123
- 5-HT₃ receptors
 - activation by antidepressant, 26
 - CCK immunoreactivity, 12
 - densities of binding sites, 12
 - and dopamine function, 21
 - DA release, 22
 - effects of nicotine, 373–374
 - in nigro-striatal DA system, 47
 - receptor antagonists, 22, 58
- 5-HT₆ receptors
 - antagonist, 189
 - APDs action, role in, 188–189
 - gene polymorphism (C267T) with clozapine in schizophrenic patients, 208
 - like immunoreactivity, 215
 - localization of, 188
 - SB-271046, antagonist, 189
 - SB-399885, antagonist, 189
 - SB 258510A, antagonist, 189
- 5-HT₇ receptors
 - mRNA expression of, 13
 - VTA and SNc DA neurons, firing and burst rate, 58
- 5-HT receptor subtypes
 - and effects of nicotine, 368–375
- 5-HT releasers, potential adverse effects of, 392–397
- 5-HTT, serotonin transporter, 86–88
- HUMTH01 microsatellite, 79–80
- 6-hydroxydopamine (6-OHDA), 49, 429
- 5-hydroxytryptamine, 605
- 5-hydroxytryptamine receptor 2A (HTR2A)
 - A1438AG polymorphism, 83–84
- 5-hydroxytryptophan, 126
- hyperpolarization, 610
- hyperpolarization-activated cation current (I_h), 50, 60–62
- hyperprolactinemia, 120
- ICS205930, receptor antagonists, 22
- ICV, *see* intracerebroventricular (ICV)
- IL, *see* infralimbic (IL)
- iloperone, 16
- indexing, attentional deficit, 520–521
- infralimbic (IL), 584
- inhibitory postsynaptic currents (IPSC), 437
- inhibitory postsynaptic potential (IPSP), 61

- initial segment-somatodendritic (IS-SD) break, 49
- inositol phosphate (IP3) receptors, 585
- insomnia, 637
- interspike interval (ISI), 50
- in VTA, 272–273, 274–275, *see also* cell firing, in VTA
- mathematical description of, 278–279
- intertrial interval (ITI), 521
- intracerebroventricular (ICV), 568
- inverse agonism, *see also* constitutive receptor activity
- at 5HTA_{2A} receptors, 290, 294
- at 5HTA_{2C} receptors, 288
- ion-gated channel signal transduction (5-HT₃), 122
- IP3, *see* inositol phosphate (IP3) receptors
- iproniazid antidepressant, 246
- ipsapirone, antidepressant agents, 26
- IPSC, *see* inhibitory postsynaptic currents (IPSC)
- IPSP, *see* Inhibitory postsynaptic potential (IPSP)
- ISI, *see* Interspike interval (ISI)
- IS-SD, *see* Initial segment-somatodendritic (IS-SD) break
- ITI, *see* intertrial interval (ITI)
- ketamine, 179, 184
- large aspiny interneurons (LAI), 433
- latent inhibition (LI), 189
- L-DOPA, 465, 573, 588, 637
- L-DOPA-induced dyskinesia (LID), 437, 444–445, 480
- learning process
- in DA, 611–613
- dendritic spines, 613–616
- in 5HT, 607–611
- 5-HT-DA interaction, 616–617
- levodopa (L-dopa), *see* L-dopa
- 3L4F, 410–411, *see also* Tat-3L4F
- L_G allele, 86
- LI, *see* latent inhibition (LI)
- LID, *see* L-DOPA-induced dyskinesia (LID)
- lidocaine, 583
- ligands, 5-HT receptors, 333–336
- locomotor activity
- cocaine-induced behaviour and, 352–353
- dopamine autoreceptors, 350–351
- serotonin autoreceptors, 350–351
- locomotor hyperactivity
- 5-HT_{2A} receptor, 326–329
- 5-HT_{2C} receptor, 326–329
- long-term depression (LTD), 473, 574
- long-term memory (LTM), 570, 607
- long-term potentiation (LTP), 473, 574, 613
- loxapine, 16, 156, 159–163, 165–169
- LSD, *see* lysergic acid diethylamide (LSD)
- LTD, *see* long-term depression (LTD)
- LTM, *see* long-term memory (LTM)
- LTP, *see* long-term potentiation (LTP)
- L-tryptophan, 390
- lysergic acid diethylamide (LSD), 118–119, 127, 184
- M100907, 5-HT_{2A} antagonist, 181, 183, 185
- magnesium, 614
- magnetic resonance imaging (MRI), 496
- major depression, 246
- MAO-B, *see* monoamine oxidase (MAO-B)
- MAOI, *see* monoamine oxidase inhibitors (MAOI)
- MAP kinase, *see* mitogen activated protein (MAP) kinase
- mazindol, 22
- m-chlorophenylpiperazine (mCPP), 392
- MDA, *see* 3,4-methylenedioxymphetamine (MDA)
- MDL 100907, antipsychotic antagonist, 120, 123–127, 124, 125, 131, 184
- MDMA, *see* 3,4-methylenedioxymphetamine (MDMA)
- medial ascending pathway, 238
- substantia nigra and caudate-putamen, 239
- medial prefrontal cortex (mPFC), 8, 11, 13–22, 24, 27–28, 48, 55, 59, 124
- medial raphe nucleus (MRN), 3, 46, 236, 424, 568
- electrical stimulation on DA neuron activity, 53
- median raphe (MR), 9
- medication show signs, in ADHD, 554–557
- medium spiny neurons (MSN), 427
- melperone, 16
- memory formation, 606
- memory process
- in DA, 611–613
- dendritic spines, 613–616
- in 5HT, 607–611
- 5-HT-DA interaction, 616–617

- mesocortical dopamine, 124–125
 - mesocortical dopaminergic pathway from VTA, 219
- mesolimbic dopamine, 123–124
 - in depression
 - animal models, 266–268
 - and burst influence, 278–279
 - in humans, 266
 - and ISI, 278–279
 - neuronal activity, in rats, 272–273
 - VTA, *see* ventral tegmental area (VTA), in depression
 - mesolimbic pathway in ventral tegmental area (VTA) and nucleus accumbens (NAc), 214
- mesolimbic system, 568
- mesostriatal system, 568
- metabotropic glutamate receptor-mediated inhibition postsynaptic current (mGLUR-IPSC), 61
- metabotropic glutamate receptor(mGLUR), 61–62
- 4-methyl-2,5-dimethoxyamphetamine (DOM), 577
- methylenedioxyamphetamine (MDA), 237
- 3,4-methylenedioxyamphetamine (MDA), 577
- 3,4-methylenedioxymethamphetamine (MDMA), 577
- methylphenidate, 544, 554
 - as antidepressant, 266
 - DA elevating agents, 221
- methysergide, 392, 394
- mGLUR, *see* metabotropic glutamate receptor (mGLUR)
- mGLUR-IPSC, *see* metabotropic glutamate receptor-mediated inhibition postsynaptic current (mGLUR-IPSC)
- mianserin, tricyclic antidepressants, 25
- microdialysis, 8, 587
 - application of, 13
 - effects on neurotransmission, 13
 - probe, 14
- mirtazapine, antidepressant agents, 26
- mitogen activated protein (MAP) kinase, 185
- MK 212, 5HT_{2A/2B/2C} receptor agonists, 57
- MK-801-induced hyperlocomotion [an N-methyl-D-aspartate (NMDA) antagonist], 125
- p*-MMPI, 124
- modafinil DA elevating agents, 221
- monoamine, 550
 - neurons, 386
- monoamine oxidase inhibitors (MAOI), 213
- monoamine oxidase (MAO-B), 550
 - MAOB enzyme and serotonin, 246
 - MAO enzymes isoforms, 219–220
 - monoamine hypothesis of depression, 219
 - monoamine oxidase A (MAOA) gene
 - fragment polymorphisms, 84
 - gene–environment study, 85
 - G x E interaction, 85
 - transfection studies, 84
- monoamine transporters in psychiatric disorders, 220
- Morris water maze, 570, 610
- motor activity, 604
- motor tics, 5
- mPFC, *see* Medial prefrontal cortex (mPFC)
- MRN, *see* Medial raphe nucleus (MRN)
- mRNA editing, 5-HT_{2C} receptors and, 289
- MSN, *see* medium spiny neurons (MSN)
- NAA, *see* N-Acetyl aspartate (NAA)
- NAC, *see* Nucleus accumbens (NAC)
- N-Acetyl aspartate (NAA), 82
- NADPH diaphorase activity and serotonin-immunoreactivity colocalization, 244
- National Institute of Mental Health (NIMH), 157
- NA transporter, 554
- NBM, *see* nucleus basalis magnocellularis (NBM)
- N-Desmethylozapine (NDMC), 179
- NDMC, *see* N-Desmethylozapine (NDMC)
- nefazodone antidepressant drug, 223
- neostriatum, 611
- nerve cells, 603
- neural mechanisms, underlying LID, 480
- neural oscillation model, of basal ganglia function, 500, *see also* tourette syndrome (TS)
- neurobiology, 551
- neurochemical models, of TS, 500–507, *see also* tourette syndrome (TS)
- neurochemical modulation
 - 5CSRTT, 523–526
 - PFC, 520
 - SSRT, 533–534
- neurochemistry, 551–552
- neurogenesis, 245–246
- neuroimaging, 550
- neuroleptic drugs, 103
- neuromodulation, 568

- neuron, 568
- neuronal cytoarchitectonic pattern, 605
- neuronal firing, *see* cell firing
- neuroplasticity, 185, 245
 - in ageing brain, 250–251
 - depression and, 246–248
- neuroprotection, 185
- neuropsychiatric disorders, 3, 145
 - in attentional processes, 518–519, *see also*
 - attentional processes
 - ADHD, 519
 - schizophrenia, 518–519
 - subcortical dementias, 518
- neuropsychology, 552–554
- neuroserpin, 251
- neurotoxin-provoked cell, 631
- neurotransmitter function, 424
- neurotransmitter systems, 567, 605
 - roles of, 626–627
- nicotine
 - behavioural features of, use, 362–363
 - mechanisms of action of, in smoking cessation, 365–366
- nicotine in smoking cessation
 - mechanisms of action of, 365–366
 - 5-HT receptor subtypes, 368–375
 - potential for 5-HT receptors, 366–367
- nicotinereplacement therapy (NRT), 362
- nigral cell, 572
- nigrostriatal dopamine, 123
 - nigrostriatal system, 5-HT_{2C} receptors impact, 123
- nigrostriatal system, 555
- NIMH, *see* National Institute of Mental Health (NIMH)
- NIMH Psychopharmacology Drug Screening Program, 178–179
- NK1 antagonists, anxiolytic effects, 250
- NMDA, *see* N-methyl-D-aspartate (NMDA)
- N-methyl-D-aspartate (NMDA), 522, 585
- N-methyl-D-aspartate (NMDA) receptors, 614
 - antagonist, 181, 480
 - ketamine, 184
 - PCP and MK-801, 180, 185
 - phencyclidine, 184
- channel blocker
 - GABA release, 18
 - induced DA release, 18
- nomifensine, as antidepressant, 266
- non-clock-like neurons, 235
- non-selective MAO inhibitors in psychiatric and neurological disorders, 220
- non-serotonergic CP-projecting neurons, in caudal parts of ventromedian and dorsomedian DRN, 238
- noradrenaline and 5-HT deficits in neurotransmission, 219
- noradrenalin (NA), 544
- NO synthesizing neurons, 244–245
- NS-2330, 449
- nucleus accumbens (NAc), 179–180, 184, 186–189, 236, 267, 280–281, 408
 - and serotonin–dopamine, 268–270
- nucleus basalis magnocellularis (NBM), 569
- nucleus raphe magnus (NRM), 424
- nucleus raphe obscurus (NRO), 424
- obsessive compulsive behaviour (OCB), 496
- obsessive compulsive disorder (OCD), 86, 496, 531
- OCB, *see* obsessive compulsive behaviour (OCB)
- occipital cortex, 612
- OCD, *see* obsessive compulsive disorder (OCD)
- OCD treatment, in TS, 504–505
- odds ratio (OR), 77
- 6-OHDA, *see* 6-hydroxydopamine (6-OHDA)
- olanzapine, antipsychotic drugs, 16, 19, 27, 83, 104, 109, 110, 120, 126–128, 130, 143, 178–189, 216
- oligodendrocytes, 555
- ondansetron, 22
- operant behaviour, 606
- OR, *see* odds ratio (OR)
- orexinergic cell, 629
- PA, *see* passive avoidance (PA)
- PAG, *see* phosphate-activated glutaminase (PAG)
- PAL-287, 297–299
- PANSS, *see* Positive and Negative Syndrome Scale (PANSS)
- parabrachial nucleus (PBN), 236
- para-chlorophenylalanine (PCPA), 51, 53–55, 568
- p*-Chloroamphetamine (PCA), 237
- paranoid psychosis, 213
- paraventricular nucleus (PVN) of thalamus, 236
- Parkinsonian resting tremor, 445

- Parkinson's disease (PD), 424, 572, 626, 637
 neuropsychiatric disorders, 5, 7, 128
 antidyskinetic actions, 29
 symptoms, 29
- paroxetine, 268–269
 antidepressants, 26, 51–52
- partial agonists at postsynaptic receptors, 634
- parvalbumin (PV) cells, 103, 107, 108, 109
- passive avoidance (PA), 570
- pathological studies, of TS, 496–498, *see also*
 tourette syndrome (TS)
- PCA, *see* *p*-chloroamphetamine (PCA)
- p*-chloroamphetamine (PCA), 569
- PCP, *see* Phencyclidine (PCP)
- PCPA, *see* para-chlorophenylalanine (PCPA)
- PD, *see* Parkinson's disease (PD)
- PDSP, *see* Psychoactive Drug Screening Program (PDSP)
- pedunculopontino nucleus (PPN), 424
- perceptual memory, 607
- pergolide, 632, 633
 dopaminergic receptor agonists, 221
- Perphenazine, 156, 159–163, 165–169
- PET, *see* positron emission tomography (PET)
- PFC, *see* prefrontal cortex (PFC)
- phencyclidine (PCP), 126–127, 179–181, 186, 188, 522
- phenothiazine imipramine drug, 213
- phenylethanolamine-N-methyltransferase (PNMT)-immunoreactive neurons and PAG, 244
- phosphatase and tensin homologue deleted on chromosome 10 (PTEN), 409
 association with 5-HT_{2c} receptor, 409–412
 -5-HT_{2c} receptor complex, location in VTA, 415–418
 similarity and differences with 5-HT_{2c} receptor, 414–415
 uncoupling to 5-HT_{2c} receptor, 412–414
- phosphatase and tensin (PTEN) enzyme
 protein phosphatase activity, 4
- phosphate-activated glutaminase (PAG), 244
- phosphoinositolmediated signal transduction (5-HT_{2A/2B/2C}), 122
- phospholipase A₂ (PLA₂), 48
- phospholipase C (PLC), 433
 and 5-HT_{2C} receptors, 48
- phyletic memory, 606
- picrotoxin, 586
- pimavanserin, 5-HT_{2A/2C} selective antagonist, 183
- pimozide, 157–163, 165–169
- pitrazepin, GABA-A antagonist, 181
- PLC, *see* phospholipase C (PLC)
- PNMT, *see* phenylethanolamine-Nmethyltransferase (PNMT)-immunoreactive neurons and PAG
- polymerase chain reaction (PCR), 428
- polymorphic cells, 610
- polysomnographic studies, 638
- Positive and Negative Syndrome Scale (PANSS), 183
- positron emission tomography (PET), 123, 427, 496, 545
- post-synaptic alterations, of dyskinesias, 473,
see also dyskinesia, L-DOPA-induced
- postsynaptic bioelectric effect, 605
- postsynaptic receptors, 634
- PPI, *see* pre-pulse inhibition (PPI)
- pramipexole dopaminergic receptor agonists, 221
- prazosin, alpha1-adrenergic antagonist, 181
- preclinical studies
 of 5-HT_{1A} ligands, 482–486
 of SSRI, 490
- prefrontal cortex (PFC), 124, 518, 568, 584–588, 609, *see also* attentional processes
 anatomical substrate, of attention, 519–520
 cell types in, 103
 cortical areas, 102
 cortico-cortical connectivity in, 102, 103
 and DA, 584–586
 divisions of, 101
 and 5HT, 586
 and 5-HT/DA interaction, 586–588
 inhibitory neurons, role of, 103
 neurochemical modulation, 520
- prefrontocortical neurons, 607
- prepro-galanin mRNA, DRN neurons levels in, 244
- pre-psychotic prodrome, 142
- pre-pulse inhibition (PPI), 127, 180–181
- pre-synaptic model, of dyskinesias, 468–470,
see also dyskinesia, L-DOPA-induced
- presynaptic receptors, 634
- procedural memory, 606
- prolactin secretion, 183
- prolong HRT, 125

- protein kinase B (Akt) pathways, 185
- psychiatric complications, 445–446
- psychiatric diseases., 588
- Psychoactive Drug Screening Program (PDSP), 157
- psychoneural effect, 605
- psychoneural processes, 607
- psychosis prevention
- and animal developmental models, 146
 - dopamine receptors as pharmacological targets, 145
 - medicine preventive interventions
 - primary, 142
 - secondary, 142
 - tertiary, 142
 - neonatal hippocampal lesion model,
 - pathophysiology of, 147
 - psychotropic drugs, 3, 5
 - serotonin and dopamine, pharmacological role in, 143
 - studies in, 142–143
 - primary prevention treatments, 142
- psychostimulant abuse, and 5HT receptors, 326, 327
- animal models, 326, 327
 - CPP, 330–332
 - drug discrimination, 329–330
 - locomotor hyperactivity, 326–328
 - mesocorticoaccumbens pathway, 328–329
 - self administration of drugs, 330–332
- psychotic disorders, 141, 142, 143, 156
- PTEN, *see* Phosphatase and tensin homologue deleted on chromosome 10; Phosphatase and tensin (PTEN) enzyme
- putative dopamine and serotonin interactions, in ADHD, 548–549
- PV, *see* Parvalbumin (PV) cells
- pyramidal cells, 10, 587, 609
- pyramidal neurons, 609
- quantitative autoradiographic mapping, 47
- quetiapine drug, 16, 104, 120, 126, 128, 130, 178, 182–183, 185–187
- ‘fast-off’ drug, 204
 - transient occupancy of brain DA D₂ receptors, 216, 223
- quinpirole, 581
- raclopride, D2 receptor antagonist, 181
- raphe nuclei (RN), 568
- raphe stimulation and activity of dopaminergic neurons, 122
- rapid-eye-movement sleep (REMS), 626
- Rauwolfia alkaloid reserpine and depression, 213
- Receptor Selection and Amplification Technology (R-SAT), 181
- receptors’ expression, in animal models, 442–444
- REMS, *see* rapid-eye-movement sleep (REMS)
- reuptake inhibitors, 386–387
- reversible MAO-A inhibitors (RIMAs), 220
- RIMAs, *see* Reversible MAO-A inhibitors (RIMAs)
- risperidone, antipsychotic drugs, 16, 19, 120, 126–128, 141–144, 147–148, 178–187, 189, 201, 216
- ‘slow-off’ drug, 204
- ritanserin, 19, 120, 123–126, 124, 125, 128, 129, 131
- 5-HT_{2A} receptors blockage, 56
- RN, *see* raphe nuclei (RN)
- RO 60-0175, selective 5-HT_{2C} receptor agonist, 57
- Ro600175, 408
- R-SAT, *see* Receptor Selection and Amplification Technology (R-SAT)
- SB 206553, 5-HT_{2C/2B} receptor antagonist, 57
- SB 242084, selective 5-HT_{2C} receptor antagonist, 57
- schizophrenia, 518–519, 584, 637
- schizophrenia, neuropsychiatric disorders, 8–9, 27, 80, 101, 104, 107–110, 117, 121, 125, 142–148, 179–186, 188–189
- cortical glutamatergic pyramidal neuron, role of, 110
- DA D2 receptors, blocking, 28
- dopamine and, 118
- GABAergic interneurons and, 103
- 5-HT, dopamine and receptors in, 109–111
- 5-HT_{1A} receptor, monoaminergic activity., 29
- HTR_{2C} gene in, 187
- 5-HT2 receptor antagonism, importance, 27
- mesocortical DA system, hypofunction, 27
- PFC region and, 110
- serotonin, role of, 105, 118–119
- symptoms and side effects, 118
- scopolamine, 578

- SD 5-HT_{1A} autoreceptors, 55
- second-generation antipsychotic drugs (SGAs), 119, 200
- 5-HT receptor subtypes, 204, 208
- PET studies of, 202
- prefrontal dopamine release and, 205
- serotonin–dopamine interaction role in, 208
- secretin gastrointestinal peptide in DRN, 245
- selective serotonin reuptake inhibitor (SSRI), 5, 25, 51, 88, 126, 434, 482, 497, 634–635
- anxiety and depressive disorders, 221
- clinical studies, in LID, 490
- effect on smoking behaviour in humans, 367–368
- and neurogenesis, 246
- preclinical studies, in animal models, 490
- SSRI fluvoxamine drug, 253
- SSRI paroxetine drug, 254
- selegiline MAO-B inhibitor, 220
- seroquel antipsychotic drug, 223
- serotonergic afferents, influence of, 630
- serotonergic cells, 627
- serotonergic manipulation effects
- 5CSRTT, 523–525
- set-shifting, 530–531
- SSRT, 534
- serotonergic nerve terminals, 605
- serotonergic nuclei, 629
- serotonergic receptors and cognition, 575–579
- serotonergic system, 568
- serotonin–dopamine interactions, 3, 7
- anatomical sites of, 4
- aspect of, 4
- clinical evidence, 128
- cognition and, 127
- electrophysiological evidence, 45
- extrapyramidal symptoms (EPS) and, 126
- 5-HT receptors mediating dopaminergic function, 222–223
- and monoamine oxidase (MAO) enzymes, 219–220
- and monoamine transporters, 220–222
- negative symptoms, 126
- and neuropsychiatric disorders, 24
- depression, 24
- drugs of abuse, 30
- Parkinson's disease, 29
- schizophrenia, 27
- psychiatric disorders, 213
- serotonin–dopamine hypothesis, 200–201
- system, 214
- in therapeutic agents for
- depression and anxiety, 219
- schizophrenia, 215–216
- serotonin/dopamine receptors, 214
- serotonin (5-HT), 118–119, 178, 185, 347–348, 543, 545–548, *see also* 5-HT
- in aggression, 308–309
- autoreceptor agonists, 467
- in behavioural state modulation, 633–637
- 5-HT_{1A} receptor and sleep variables, 634–635
- in brain, 348–349
- and autoreceptors, 349–350
- in central dopamine (DA) systems, 3
- and cocaine-induced behaviour, 352–354
- DA neuron activity
- modulation of, 49
- serotonergic control of, 54–59
- depolarization, 61
- and depression, 246
- dopamine bioavailability, 270–272
- in nucleus accumbens (NAc), 268–270
- in depression, 309–310
- dopaminergic function, control of, 7
- in drug addiction, 61
- GABA_B synaptic potential, 61
- G-protein coupled receptors, 9
- immunoreactive cell bodies in rat brain, 46
- immunoreactive fibres, 236–237
- immunoreactivity colocalization with NO synthase (NOS), 244
- interaction and pathways, 122–125
- in investigations of ADHD, 549–554
- genetics, 549–551
- neurochemistry, 551–552
- neuropsychology, 552–554
- mGLUR-IPSC inhibition, 61
- microiontophoretic application, 49–50, 53
- pre-clinical behavioural evidence, 125
- quantitative autoradiographic mapping in rat brain, 47
- receptors, 12–13, 122, 143–144, 156, 166, 167, 172, 629–630
- blockade and subtypes, 4
- in central nervous system (CNS), 8
- class, 9–10

- in CNS, anatomical distribution of, 47
- and depression, 248
- in dopaminergic activity, 4
- 5-HT_{2A}, 141
- 5-HT_{2C}, 155, 156
- 5-HT₂ receptor family, 11
- 5-HT₁ receptors class, 10
- localization, 9
- monkey PFC and, 104–105
- regulate DA neuronal activity in brain, 5
- serotonin_{2A}, 185
- subtypes in, 104
- subtypes localization, 63
- serotonergic neurons and plasticity, 245
- in sleep and waking regulation, 639–640
- and spontaneous behaviour, 350–352
- in suicidal behaviour, 309–310
- in TS, 504
- serotonin-rich grafts, 482
- serotonin system, in dyskinesias
 - human, observations on, 471–473
 - MPTP-lesioned monkeys, observations on, 470–471
 - rats, observations on, 466–468
- serotonin transporter (SERT), 74–75, 88, 490
 - amygdala neuronal activity, 87
 - expression of, 237
 - 5-HTTLPR polymorphism, 87
 - polymorphisms and anxiety-related features, 249
 - short and long allele, 86
 - SLC6A4, 86–88
- SERT, *see* serotonin transporter (SERT)
- sertindole drug, 109, 120, 204
- sertraline, 51–52
 - chronic treatment in, 26
- setoperone, 128–129
- set-shifting, attentional processes, 526–532
 - clinical implications, 531–532
 - fronto-striatal lesion effects, 526–528
 - neurochemical modulation, 528–531
 - DA-5-HT interaction, 531
 - dopaminergic manipulation effects, 528–530
 - serotonergic manipulation effects, 530–531
- SGAs, *see* second-generation antipsychotic drugs (SGAs)
- short-term memory (STM), 569, 607
- side effects, extrapyramidal, 446–449
- Simian virus 40 (SV40), 78
- single nucleotide polymorphisms (SNP), 74, 550
- single photon emission tomography (SPECT), 502
- sleep in patients
 - with Parkinson's disease, 637
 - with schizophrenia, 637–638
- sleep variables, 634–635
 - full agonists autoreceptors, 634
 - partial agonists at postsynaptic receptors, 634
 - postsynaptic receptors, 634
 - presynaptic receptors, 634
 - serotonin reuptake inhibitors, 634–635
- slow wave sleep (SWS), 626
- smoking cessation
 - mechanisms of action of nicotine in, 365–366
 - nicotine use on the potential for 5-HT receptors, 366–367
 - nicotinic strategies for, 362
 - role of animal models, 363–364
- SN, *see* substantia nigra (SN)
- SNc, *see* substantia nigra pars compacta (SNc)
- SNP, *see* singlenucleotide polymorphisms (SNP)
- SNpc, *see* substantia nigra pars compacta (SNpc)
- SNr, *see* substantia nigra reticulata (SNr)
- SNr activity, in 5-HT modulation, 434–436
- somnolence, 638
- spiperone, APDs, 187
- spontaneous behaviour, 350–352
- sprouting, 245
- SR 46349B, 5-HT_{2A/2C} selective antagonist, 123, 183
- SSRI, *see* selective serotonin reuptake inhibitor (SSRI)
- SSRT, *see* stop-signal reaction time (SSRT)
- standard model, of basal ganglia function, 498–499, *see also* tourette syndrome (TS)
- stimulant addiction, dual deficit model of, 387–389, 388–389, 392–394, 397
- stimulant effects of DA release, 5HT and, 389–382
- stimulants, 385–386
- stimulus, 580
- STM, *see* short-term memory (STM)
- STN, *see* subthalamic nucleus (STN)
- STN activity, in 5-HT modulation, 436–439
- stone test, 570
- stop-signal reaction time (SSRT), 521, 532–534
 - fronto-striatal lesion effects, 532–533
 - neurochemical modulation, 533–534
 - dopaminergic manipulation effects, 533
 - serotonergic manipulation effects, 534

- striatal acetylcholine (ACh), 433
- striatal activity, in 5-HT modulation, 431–434
- striatal cognitive processes and 5-HT, 581–584
- striatal mediated cognitive processes and DA, 579–581
- striatum, in 5-HT receptor distribution, 426–429
- striatum and cognitive processes, 579–584
 - striatal cognitive processes and 5-HT, 581–584
 - striatal mediated cognitive processes and DA, 579–581
- striosome and matrix model, of basal ganglia
 - function, 499–500, *see also* tourette syndrome (TS)
- subcortical dementias, 518
- subiculum, 610
- Substance P, 244
- substantia nigra pars compacta (SNc), 181, 214, 465
 - DA-containing neurons characteristics, 49
 - striatal DA source, 9
- substantia nigra pars compacta (SNpc), 588
- substantia nigra pars reticulata (SNr), 46
 - GABA-ergic neurons, 9
- substantia nigra reticulata (SNr), 425
- substantia nigra (SN), 3, 46, 424, 568
 - compartments, 9
 - in 5-HT receptor distribution, 429–431
- substrate-type releasers, 387
- subthalamic nucleus (STN), 29, 424, 533
 - in 5-HT receptor distribution, 431
- suicidal behaviour, 308
 - dopamine in, 309–310
 - serotonin in, 309–310
- sulpiride, 633
- sumatripan, 552
- SV40, *see* Simian virus 40 (SV40)
- SWS, *see* slow wave sleep (SWS)
- synaptic stimulus, 615
- synaptogenesis, 245
- Tardive dyskinesia, 27, 130
- Tat-3L4F, 411–412, *see also* 3L4F
 - interfering peptide, 413
- Δ^9 -tetrahydrocannabinol (THC), 5, 412
- TFMPP, *see* Trifluoromethylphenylpiperazine (TFMPP), 5-HT_{1B} receptor agonists
- TH, *see* Tyrosine hydroxylase (TH)
- thalamic nuclei, 616
- THC, *see* Δ^9 -tetrahydrocannabinol (THC)
- thiothixene and thioridazine drug, 178, 187
- T-maze, 569, 573
- tonic spike firing, 121
- tourette syndrome (TS)
 - anatomic localization, 496
 - centre-surrounded model, of basal ganglia function, 499
 - defined, 495
 - dopamine-serotonin interactions, 506–507
 - neurochemical models, 500–507
 - OCD treatment, 504–505
 - pathological studies, 496–498
 - serotonin, 504
 - standard model, of basal ganglia function, 498–499
 - striosome and matrix model, of basal ganglia function, 499–500
 - treatment, 501–502
- tranylcypromine, 554
- trifluopromazine, APDs, 27
- trifluoromethylphenylpiperazine (TFMPP),
 - 5-HT_{1B} receptor agonists, 20, 55
- trigeminal somatosensory pathway, 236
- Trp, *see* tryptophan (Trp)
- tryptophan depletion, 555
- tryptophan hydroxylase 2 and depression, 249
- tryptophan (Trp), 569, 607
- TS, *see* tourette syndrome (TS)
- TS treatment
 - and DA, 501–502
 - and 5-HT, 505–506
- tumour suppressor enzyme PTEN, 4
- Type I and Type II (typical and atypical)
 - serotonergic neurons, 235
- typical antipsychotic medications, 168, 171
 - 5-HT_{2C} and D₂ receptor-binding affinity, 169
 - as inverse agonists, 170
- tyrosine hydroxylase (TH), 47–48, 62–63, 79–80, 417
 - TCAT, effect on, 79–80
- unified Parkinson's disease rating scale (UPDRS), 481
- UPDRS, *see* Unified Parkinson's disease rating scale (UPDRS)

- vacuous chewing movements (VCM), 447
- valine-to-methionine (Val158Met) substitution, 80
 - BDNF-gene, 185
- Val66Met (G194A) single nucleotide polymorphism (SNP) for major depression study, 247
- Val66Met (G194A) SNP and C270T SNP within BDNF gene and major depression, 252
- valvular heart disease (VHD), 392–394
- varenicline (CHANTIX[®]), 362
- variable number tandem repeat (VNTR), 545
- vasoactive intestinal polypeptide (VIP), 244
- VCM, *see* vacuous chewing movements (VCM)
- venlafaxine, 554
- ventral ascending pathway, 239
 - amygdaloid complex and cerebral cortex, 241
 - habenula and septum, 240–241
 - hippocampus, 241–242
 - hypothalamus and thalamus, 240
 - olfactory bulb, 241–242
 - supraependymal plexus, 243
- ventral periaqueductal grey matter (VPAG), 627
- ventral tegmental area (VTA), 3, 8, 46, 102, 107, 111, 120, 124, 179, 390, 408, 568
 - DA containing neurons characteristics, 49
 - DA neurons, firing rate, 180, 187–188
 - fast Fourier transformation-based analysis, 51
 - 5HT_{2A} receptor, enhanced glutamate efflux, 18
 - 5-HT₂ receptors, auto-inhibition, 60
 - inhibition of, DA cell activity, 52
 - non-DA cells in, 12
 - parabrachial and the paranigral subdivisions of, 12
 - ventral tegmental area (VTA), in depression
 - cell firing in
 - and antidepressants, 274–275, 280–281
 - and burst influence, 277–278
 - firing patterns, 275–277
 - modes of, 273–274
 - interspike interval (ISI), 272–273, 274–275
 - mathematical description of, 278–279
- vesicular monoamine transporter 2 (VMAT2), 466
- vilazodone, 5-HT_{1A} receptor agonist and 5-HT re-uptake inhibitor, 223
- VMAT2, *see* vesicular monoamine transporter 2 (VMAT2)
- VNTR, *see* variable number tandem repeat (VNTR)
- VTA, *see* ventral tegmental area (VTA)
- WAY-100635, 5-HT_{1A} antagonist, 184
- WAY-163909, 5-HT_{2C} selective receptor agonist, 188
- WCST, *see* Wisconsin Card Sort Test (WCST)
- WIN55,212-2, 415, 417
- Wisconsin Card Sort Test (WCST), 520
- Yale Global Tic Severity Scale (YGTSS), 502
- YB-1, *see* Y box-binding protein 1 (YB-1)
- Y box-binding protein 1 (YB-1), 88
- YGTSS, *see* Yale Global Tic Severity Scale (YGTSS)
- zacopride drug, 22
- zinc finger protein (ZNF191), 80
- ziprasidone, 16, 120, 122, 126, 130, 178, 182, 185–187
- ZNF191, *see* zinc finger protein (ZNF191)
- zotepine drug, 120